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Micelar-Electrokinetic Chromatography Separation of Nitrogen-Containing Aromatic Compounds in Diesel Prepared as Microemulsion

Anastácia Silva Canto[1,2] Alessandra Licursi Maia Cerqueira da Cunha[3*] Selma Cunha Mello[4], Ricardo Queiroz Aucelio[2]

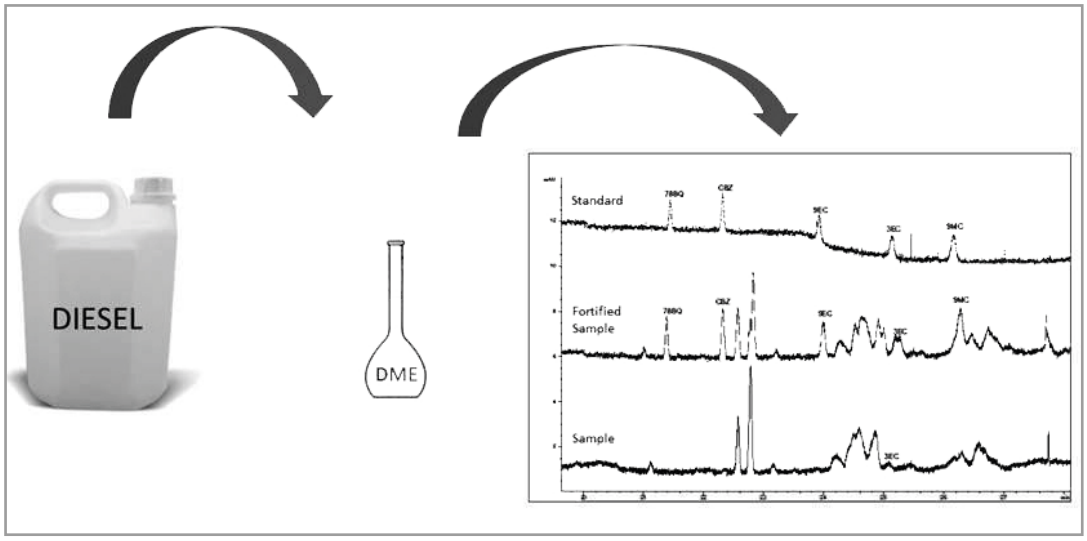
[1]Instituto de Química, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Rodovia BR 465 Km 07, Zona Rural – Seropédica, Rio de Janeiro, Brazil

[2]Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio), Rua Marquês de São Vicente, 225 - Gávea, Rio de Janeiro, Brazil

[3]Departamento de Química, Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Rua Senador Furtado, 121/125 – Maracanã, Rio de Janeiro, Brazil

[4]Centro de Pesquisas Leopoldo Américo Miguez de Mello – Petrobras, Gerência de Química, (CENPES/PDISO), Av. Horácio Macedo, 950 - Cidade Universitária, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Graphical Abstract



Steps for identification and quantification of nitrogen-containing aromatic compounds by capillary electrophoresis in diesel samples, using a stable and homogeneous microemulsion without detergent as a tool.

Nitrogen-containing aromatic compounds (NCACs) are present in petroleum fractions such as diesel. An exploratory study aiming the separation of twelve NCACs of different types (carbazoles, indoles, quinolines, acridine and aniline) by micellar-electrokinetic chromatography (MEKC) was made using micro-emulsified diesel sample and pyrrole as the internal standard. Diesel (previously dissolved in isooctane) was prepared as detergentless microemulsion and then mixed with a surfactant-containing electrolyte solution in order to prepare the microemulsion to be introduced into the capillary. The system BGE was composed by sodium dodecyl sulfate in a borate buffer (10 mmol L^{-1}; pH 9.50), containing acetonitrile and urea as chemical modifiers. Separation of the NCACs was achieved using a gradient of applied voltages (10 kV up to 20 min and then 30 kV up to the end of the run) at 15 ºC. Instrumental limits of detection (LOD) varied from 0.7 to 4.9 mg L^{-1} depending upon the analyte. LOD in diesel samples depend upon the dilution factor. Diesel samples (fortified with 12 NCACs) were analyzed,

enabling recoveries varying from 85 to 106%. Commercial diesel samples were analyzed and some NCACs were detected in some of the samples and effectively quantified (most carbazoles) in two of them at levels varying from 2.4 to 11.8 g L^{-1} level. The study has shown the possibility of separating NCACs directly in complex diesel samples providing a way to screening samples for the ones presented at g L^{-1} levels.

Keywords: Diesel; Microemulsion; Nitrogen-containing aromatic compounds; Micellar-electrokinetic chromatography.

INTRODUCTION

Petroleum is the major energy source in the world and it is the prime raw material for a wide range of products, including fuels such as gasoline and diesel. Among the most relevant contaminants in petroleum are sulfur, oxygen and nitrogen containing compounds [1-3]. In most advanced countries, environmental law severely restric the sulphur content in petroleum derivatives, but for nitrogen-containing compounds, the critical limit is mostly based on information about the long-term stability of the final products rather than based on concerns about emission of pollutants. Little is availabe about the nitrogen-containing aromatic compounds (NCACs).

Nitrogen-compounds in crude oil accounts for 0.1 to 2% (w/w) of whole composition. Even at such concentrations they poison cracking catalysts, induce the formation of gum in fuels and produces nitro-compounds and NO_X due to incomplete combustion [4]. A fraction of the nitrogen-compounds that compose petroleum are NCACs that concentrate in the higher boiling fractions of petroleum distillates. These substances are generally classified as basic (pyridine, quinoline, indoline and benzoquinoline) and non-basic (pyrrole, indole, carbazole and benzocarbazole) [5,6].

Scientific efforts have been made to screen, identify, quantify and remove NCACs from petroleum derivatives. There are several methods developed for the qualitative detection of NCACs, also for their quantitative determination, varying from those used for screening samples, for identification or quantification of NCACs grouped into classes, to those more sophisticated able to quantify individual isomers. The determination of non-basic nitrogen compounds in gasoline and in diesel was accomplished by differential pulse voltammetry, using a glassy carbon electrode and after solid phase extraction (SPE) allowing limits of detection (LOD) in the μg L^{-1} [7,8]. Quinolines, carbazoles, indoles and anilines along other NCACs were identified by mass spectrometry (MS) in extracts of residual fluid catalytic cracking diesel after column chromatographic separation of the sample fractions [9]. A qualitative evaluation using gas chromatography (GC) coupled to MS finding quinoline, indole and carbazole derivatives in atmospheric gas oil while aniline, indole and carbazole derivatives were found in light cycle oil [10]. GC determination of methylbenzo[c]acridines were achieved after oxidation to formylbenzo[c]acridines followed by reaction with p-fluoroaniline to form Schiff bases that were detected (down to 20 pg) by the electron-capture detector [11]. However, fuel samples were not analyzed. A fingerprinting study, involving diesel samples from three different countries, was made using sample extraction procedures and GC-MS [12]. Pyridine, pyrrole, indoles, quinolones and carbazoles were found. GC after sample extraction procedures was used with chemometrics to characterize nitrogen compounds in crude oils [13]. Two-dimensional GC with nitrogen specific detector [14-16] and with time-of-flight or quadrupole separation and MS detection [17,18] was used to characterize and quantify nitrogen compounds in diesel, heavy gas oil and middle distillate fractions using different sample extraction procedures. Sulfur and nitrogen containing aromatic compounds in shale oil were determined using two dimensional GC with a combination of four different detection systems [19]. A complex protocol was established using different sample preparation procedures depending on the target analytes (with discriminating capability for homologues). Among the NCACs found in shale oil were: indoles (1.11%), quinolines (0.60%), anilines (0.47%), acridines (0.03%) and carbazoles (0.47%).

Laser desorption ionization Fourier-transform ionic resonance cyclotron coupled with fast quadrupole

MS detector has also been used to determine nitrogen compounds in diesel fuel and in seventy Brazilian crude oil samples [20]. The basic nitrogen content varied from 0.016 to 0.151%.

Alkyl indoles, alkyl benzoquinolines and alkyl carbazoles (in the mg kg^{-1} levels) have been found in acid extracts of Brazilian diesel oil using high performance liquid chromatography (HPLC) with both MS and molecular absorption photometric detection [21,22] and by particle beam LC-MS [23]. Benzoquinolines were found to be the main NCAC group in the basic fraction, with homologues being further separated using a neutral mobile phase. HPLC with fluorimetric detection was used for the determination of six basic azaarenes in jet fuel, reaching absolute LOD values between 2 and 21 pg [24]. Neutral and basic fractions were extracted by SPE with a strong cation exchange sorbent. Oxygen and nitrogen containing aromatic compounds were determined in asphalt mixtures using HPLC-MS after sample fractionation into asphaltenes and maltenes and further liquid extraction [25]. Quinoline was quantified in the acid extract (102.2 mg kg^{-1}) and in the basic extract (63.6 mg kg^{-1}).

There is little information on the analysis of petroleum samples using capillary electrophoresis (CE) aiming NCACs. The only reported quantitative work was the one of Luz *et al.* (2014) [26] concerning the determination five acridines in diesel after SPE, using cationic solid phase, to get the analytes in acidic aqueous phase before separation by capillary zone electrophoresis, using an acidic background electrolyte, and detection by molecular absorption photometry. The concentration of the analytes into the capillary was achieved through analyte stacking, enabling LOD values at the mg L^{-1} level. For NCACs in other types of samples, two works can be identified. Guarguilo *et al.* (2000) [27] have separated indoles, carbazoles, acridines and benzoquinolines, along with 16 polycyclic aromatic hydrocarbons, in soil extracts using a capillary packed with octyldecylsilica and laser-induced fluorescence detection (LOD down to 0.4 nmol L^{-1}). Fomin *et al.* (2010) [28] identified basic NCACs in urine and blood using CE with UV absorption photometric detection but no quantitative application was accomplished.

The analysis of fuels usually requires tedious and labor-intensive procedures to separate aromatic compounds from other matrix components. Therefore, any effort to minimize complex protocols is relevant even if it involves some degradation of detection power. In this direction, the dispersion of liquid fuel samples into microemulsions brings advantages for the determination of analytes directly in samples that are immiscible with water. Due to the thermodynamic stability and homogeneity of microemulsions, prepared by mixing oily liquid and water with the aid of a surfactant and/or a co-solvent, the introduction of the oily liquid samples is facilitated and the formation of sample zones, for instance in reverse liquid chromatography, becomes feasible [29]. Sample dispersing into microemulsion reduces the organic load of the analyzed material, avoids analyte losses due to mass transferring of thick samples, minimizes sample contamination and improves the reproducibility of results since variations associated to extraction procedures are eliminated. In recent work, the analysis of diesel was accomplished by HPLC by introducing the whole diesel sample prepared as detergentless microemulsion (or DME) into the aqueous mobile phase. Ten basic and neutral NCACs were determined (fluorescence detection), at the mg L^{-1} level, in one single chromatographic run [29].

Segura-Carretero *et al.* (2000) [30] have taken advantage of microemulsions to disperse water insoluble analytes into an aqueous system with composition adjusted to enable phosphorescence measurements. A small amount of samples was prepared as microemulsions composed by a nonpolar solvent, alcohol, water and a detergent (sodium dodecyl sulfate or SDS) [30]. A few reports indicate the use of detergentless microemulsion to perform the analysis of petroleum derivatives aiming the quantification of metals and metalloids by optical emission spectrometry and voltammetry [31,32].

In the present work, a microscale study was made aiming the electrophoretic separation of 12 NCACs in diesel samples. The successful separation of carbazole (CBZ), 9-methylcarbazole (9MC), 3-ethylcarbazole (3EC), 9-ethylcarbazole (9EC), quinoline (QNL), benzo[*h*]quinoline (BhQ), indole (I), acridine (ACR), 2-methylindole (2MI), 3-methylindole (3MI), 7-methylindole (7MI) and N,N-dimethylaniline (A) (see NCACs structures in Figure 1) was accomplished using micellar-electrokinetic chromatography (MEKC) and sample directly introduced as a microemulsion to guarantee compatibility of the fuel and

the aqueous background electrolyte. No need for prior separation of basic and neutral fractions of NCACs from the sample was necessary. The screening character of the method was demonstrated in the analysis of real samples.

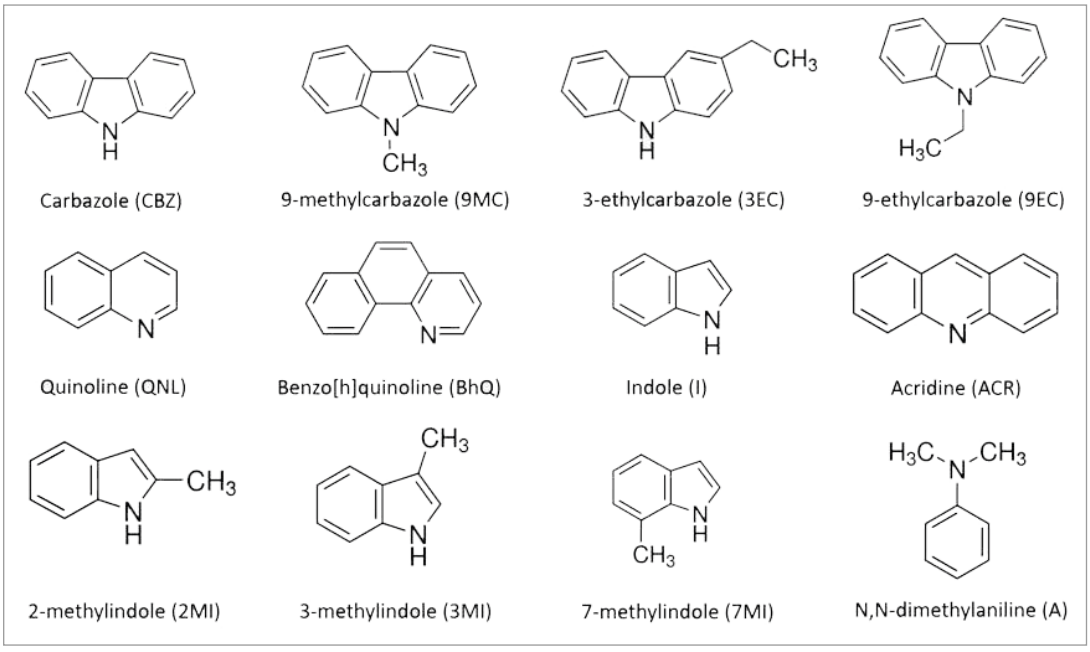


Figure 1. Molecular structures of NCACs included in this work.

MATERIALS AND METHODS

Instrumentation

Micellar electrokinetic chromatography (MEKC) was made on a HP ^{3D}CE capillary electrophoresis system (Agilent, USA) operating in normal polarity mode with a UV-vis diode array type absorption photometric detector. The pH measurements were made on a pHmeter (MS Tecnopon, model MPA-210, Brazil) with a glass membrane electrode conjugated with an Ag/AgCl(KCl_{sat}) reference electrode. A high-performance dispersion device - Ultra-Turrax IKA T18 (IKA, Brazil) - was used for the homogenization of the detergentless microemulsion. The determination of the C, H and N content in samples was made on Flash EA 1112 elemental analyzer (Thermo Electron Co). Microscopic pictures of the microemulsion was made on a Zeiss optical microscope Axio Lab.A1 (Zeiss, Germany).

Reagents and Materials

Quinoline, benzo[*h*]quinoline, carbazole, 3-ethylcarbazole, 9-ethylcarbazole, 9-methylcarbazole, indole, 2-methylindole, 3-methylindole, 7-methylindole, acridine, N-methylpyrrole and N,N-dimethylaniline were obtained from Sigma-Aldrich (USA). Sodium dodecyl sulfate (SDS), urea, boric acid, ethanol, isopropanol, acetonitrile and iso-octane (HPLC-grade) were obtained from Merck (Germany). Deionized water (resistivity of 18.2 MΩ cm) was from a Milli-Q gradient A10 ultra-purifier (Milipore, USA). Diesel and gas oil samples were obtained from Petrobras, the Brazilian Energy Company, Brazil. A fused silica capillary column (Agilent, California, USA) of 56.0 cm of length (47.5 cm of effective length) with 50 μm of internal diameter.

Solutions and Samples

Background electrolyte (BGE) solution was composed by 20% v/v of acetonitrile and 80% v/v of an aqueous solution containing H_3BO_3 (10 mmol L^{-1}), SDS 30 mmol L^{-1} and urea 2 mol L^{-1} with pH adjusted to 9.50 (with the addition of aliquots of NaOH 1 mol L^{-1}). NCACs stock solutions (1 × 10^{-3} mol L^{-1}) were

prepared in ethanol.

Standard detergenless microemulsions were prepared by the dilution of the stock analyte solutions into about 5 mL of a mixture containing isooctane (10% v/v), water (12% v/v), ethanol (55% v/v) and isopropanol (23% v/v). Appropriate aliquots (up to 50 µL) of the standard detergenless microemulsions were added to a solution consisting of 25% (v/v) ethanol and 75% (v/v) of an aqueous solution containing H_3BO_3 (5 mmol L^{-1}), SDS 20 mmol L^{-1}, urea 1 mol L^{-1} with pH 9.50 (adjusted by the addition of aliquots of 1 mol L^{-1} NaOH solution) comprising a final volume of 5.00 or 10.00 mL. This final mixture was the analysis microemulsion.

Diesel samples were previously homogenized by mechanical agitation using an Ultra Turrax (7200 rpm) apparatus during 5 min. When analyzing samples, 10 to 200 µL (depending upon the characteristics of the sample in forming stable dispersions) of a homogenized diesel sample was dissolved in isooctane comprising about 1 to 6 mL total volume) then mixed with the appropriate volumes of water, ethanol and isopropanol to form 5.00 mL total volume of detergentless microemulsion. Then, sample detergentless microemulsion was diluted in the appropriate aqueous solution prior to the injection into the capillary.

Capillary Electrophoresis Analysis

Freshly prepared BGE solutions were employed (changed each 5 to 10 runs). The applied potential was 10 kV from the beginning up to 20 min, increasing to 30 kV up to the end of chromatographic run. Capillary temperature was kept at 15 ºC and sample hydrodynamic injection (50 mbar) was made at 50 mbar during 10 s. The diode array UV detector was set at 230 nm.

Capillary conditioning was performed in the beginning of each working day and it consisted of three steps (each of 30 min flushing): i) sodium hydroxide 1 mol L^{-1}; ii) ultrapure water and iii) the BGE solution. Before each of the sample or standard injections, a 15 min pre-conditioning procedure was made by flushing water (4 min), sodium hydroxide 1 mol L^{-1} (4 min), water again (2 min) and BGE solution (5 min). At the end of the working day, the capillary was cleaned by flushing water (5 min), sodium hydroxide 1 mol L^{-1} (5 min), water again (5 min) then passing a flow of compressed air to dry.

Elemental Analysis and Optical Analysis

The C, H and N elemental determinations were made under He atmosphere (140 mL min^{-1}) using a thermal conductivity detector and samples placed in tin capsules. For microscopic optical pictures, a droplet of the microemulsion was placed on a glass slide.

RESULTS AND DISCUSSION

Composition and Stability of Sample Dispersions

In order to avoid any labor-intensive treatment aiming analyte separation from the sample matrix, the diesel (or gas oil) sample (typically 50 to 200 µL) was first diluted in isooctane forming a 0.5 mL solution. For standards, an aliquot of the stock standard solution, containing the analytes was directly diluted in isooctane. The dispersion made with the isooctane solution (containing either standards or samples) was stable and homogeneous (for at least 24 h) when prepared in a mixture of solvents based on the one proposed by Cunha *et al.* [29]: 0.5 mL of the isooctane NCACs solution, 0.6 mL of water, 1.1 mL of propan-2-ol and completing the final 5.00 mL volume with ethanol. This was called sample (or standard) dispersion and although microliters of it can be promptly introduced into an HPLC system [29], it could not be introduced into the capillary to perform electrophoretic analysis. Therefore, a logical strategy was to adjust the final composition of the dispersion to be introduced into the CE system (nominated as analysis dispersion) as close as possible to the BGE. In order to do that, volumes varying from 10 to 50 µL of the sample (or standard) dispersion were further dispersed in a solution containing 25% (in volume) of ethanol and 75% (in volume) of an aqueous solution containing borate buffer, surfactant and the chemical modifiers used in the system BGE (see detailed composition below), forming a final 3.00 mL analysis dispersion that was stable for at least 24 h.

Studies to Establish the Electrophoretic Separation Conditions

The starting BGE composition was borate buffer (10 mmol L^{-1} using H_3BO_3) at pH 9.50 guaranteeing (based on literature pK_a values [33-38]) that QNL (pK_a = 4.9), BhQ (pK_a = 4.7), ACR (pK_a = 5.7) and A (pK_a = 7.1) remained non-protonated while the carbazoles ($pK_a \cong 12$) the indoles ($pK_a \cong 17$) and P (pK_a = 14.2) remained protonated, in order to better interact with the pseudostationary phase (SDS organized structures) used for the MEKC separation. The BGE at pH 9.5 also contained SDS (40 mmol L^{-1}) and the chemical modifiers acetonitrile (20% in volume) and urea (2 mol L^{-1}). In these initial separation studies, standard microemulsions, initially containing a mixture of five NCACs (QNL, ACR, CBZ, BhQ and 3MI) in isooctane, were mixed with the BGE (50/50% v/v) and the NCACs final concentrations (in the analysis microemulsion) were in the 10^{-5} mol L^{-1} (equivalent to mg L^{-1} levels) in the final analysis microemulsion introduced into the capillary. Pyrrole was used as an internal standard.

The influence of temperature (25 ºC, 30 ºC and 35 ºC) was evaluated using the applied voltage of 30 kV. In all cases, the peaks were separated (baseline separation) and no significant changes in migration times were found. However, peak shape tended to be better at 25 ºC with the splitting of 3MI peak at 30 ºC and at 35 ºC and with large baseline fluctuation at 35 ºC. The electropherogram achieved at 25 ºC can be seen in Figure 2A. The applied voltage was varied from 15 to 30 kV in order to check the effect on the quality of separation at 25 ºC. In all cases, baseline separation of peaks was observed and as expected shorter migration times were found as the applied voltage was increased, with QNL (the NCAC with the faster migration velocity after P) presenting migration time (t_m) of 8.1 min with 15 kV (Figure 2B) and 3.6 min with 35 kV. The migration time interval between QNL and CBZ (NCAC with the slower migration velocity) was about 9.2 min with applied voltage of 15 kV and about 3.2 min with 30 kV.

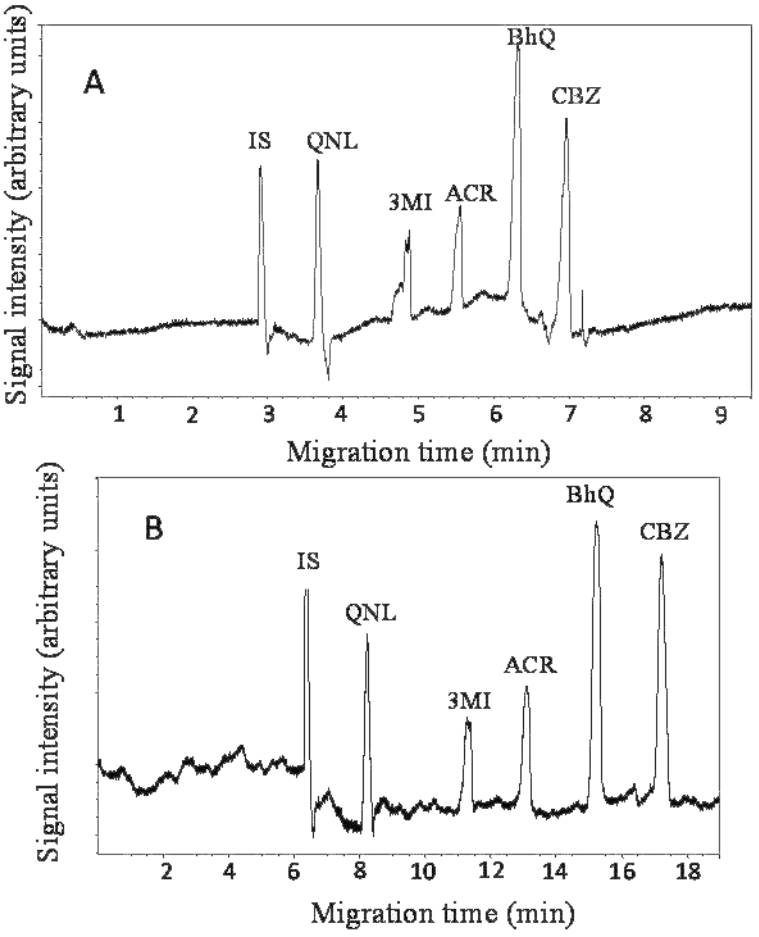


Figure 2. MEKC electropherogram of quinoline (QNL), 3-methylindole (3MI), acridine (ACR), Benzo[h]quinoline (BhQ), carbazole (CBZ) using pyrrole as internal standard (IS), sample introduction for 10 s at 50 mbar, at 25 ºC, detection at 230 nm and BGE consisting of borate buffer (10 mmol L^{-1}; pH 9.5), SDS (40 mmol L^{-1}) acetonitrile (20%) and urea 2 mol L^{-1}). A) 30 kV applied voltage; B)15 kV applied voltage.

Despite the increasing of analysis time (from 6.9 to 17.2 min, measured using the migration time of CBZ) it was decided to proceed studies using applied voltage of 15 kV as there was larger intervals between the separated peaks, leaving room to accommodate the other eight NPACs to be included in the study. Besides, at this applied voltage, peaks presented better shape and no tendency to split.

In order to decrease the somewhat larger analysis time, the concentration of SDS was adjusted from 40 mmol L^{-1} to 30 mmol L^{-1}, which decreased the migration time for CBZ from 17.2 to 12.1 min still leaving 4.3 min between migration peaks of QNL and CBZ (Figure 3A).

The next step of the study was the inclusion of the analyte A in the microemulsion introduced into the capillary. Under the conditions used to separate the first five NCACs, the peak of A appeared baseline separated from the others but with peak symmetry affected. Therefore, a decrease of temperature to 20 ºC was made in order to improve symmetry but not affecting the peak of 3MI as seen in Figure 3B. The adjustment of temperature also increased the migration time for CBZ from 12.2 to 15.1 min because of the increase of viscosity of the BGE.

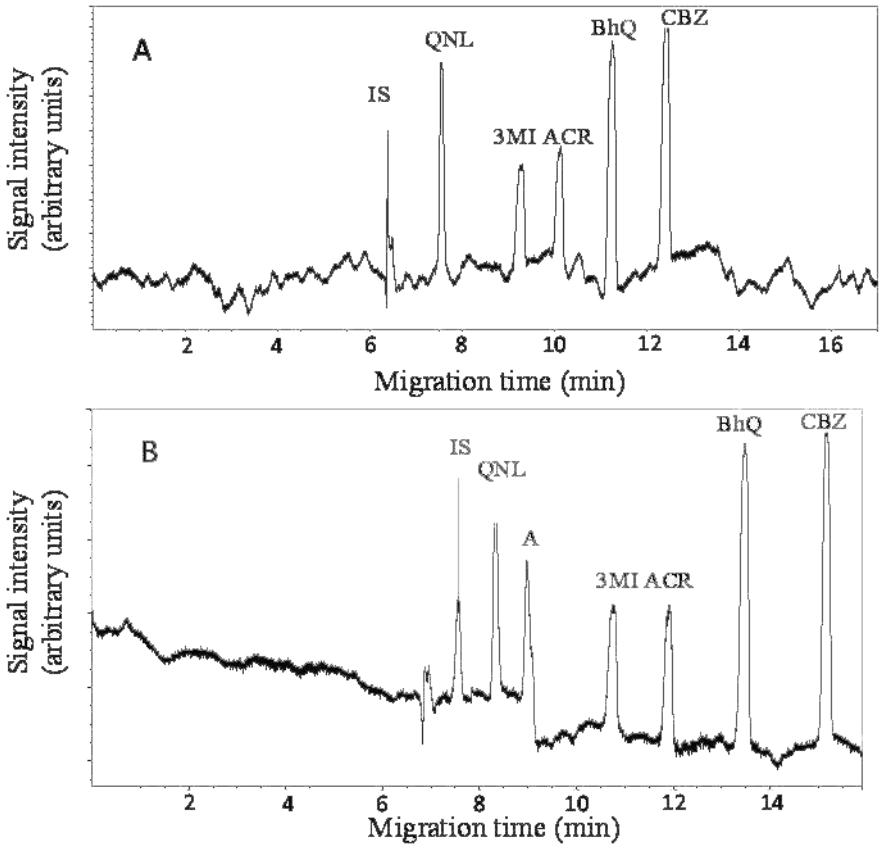


Figure 3. MEKC electropherogram of quinoline (QNL), 3-methylindole (3MI), acridine (ACR), Benzo[h]quinoline (BhQ), carbazole (CBZ) using pyrrole as internal standard (IS), sample introduction for 10 s at 50 mbar, applied voltage of 15 kV, detection at 230 nm and BGE consisting of borate buffer (10 mmol L^{-1}; pH 9.5), SDS (30 mmol L^{-1}) acetonitrile (20%) and urea 2 mol L^{-1}). A) at 25 ºC; B) at 20 ºC.

The other six NCACs (I, 2MI, 7MI, 9MC, 3EC, 9EC) were included in the micromulsion to be injected into the capillary. In the conditions already adjusted for the six NCACs, baseline separation (Figure 4A) for most of the analytes were achieved and the migration time for 9EC (the NCACs with slower migration velocity) was 23.4 min. Poor separation was achieved for the pairs of NCACs A and I and 7MI and 2MI

(detail of Figure 4), then simple adjustments of conditions were tried to improve resolution. A set of experiments were performed using microemulsion containing only these four NCACs. An improved resolution was achieved by decreasing both the applied voltage to 10 kV and the temperature to 15 ºC, achieving baseline separation for A and I and good resolution for 7MI and 2MI as can be seen in the electropherogram of Figure 4B. The use of such experimental conditions did affect neither baseline resolution between the other NCACs peaks nor the order of migration but it affected the overall analysis time by delaying the migration of 9EC (over 40 min). In order to improve analysis time, a gradient of applied voltage was used by using 10 kV from 0 to 20 min of analysis time (enough time to get the resolution for A and I and 2MI and 7MI) then increasing the applied voltage to 15 kV for the rest of the analysis. In this way, the peak of EC appeared at 37.3 min as seen in Figure 5. The conditions chosen to perform capillary introduction and electrophoretic separation of the 12 NCACs and the internal standard are indicated in Table I. Peak symmetry and resolution between peaks are presented in Table II.

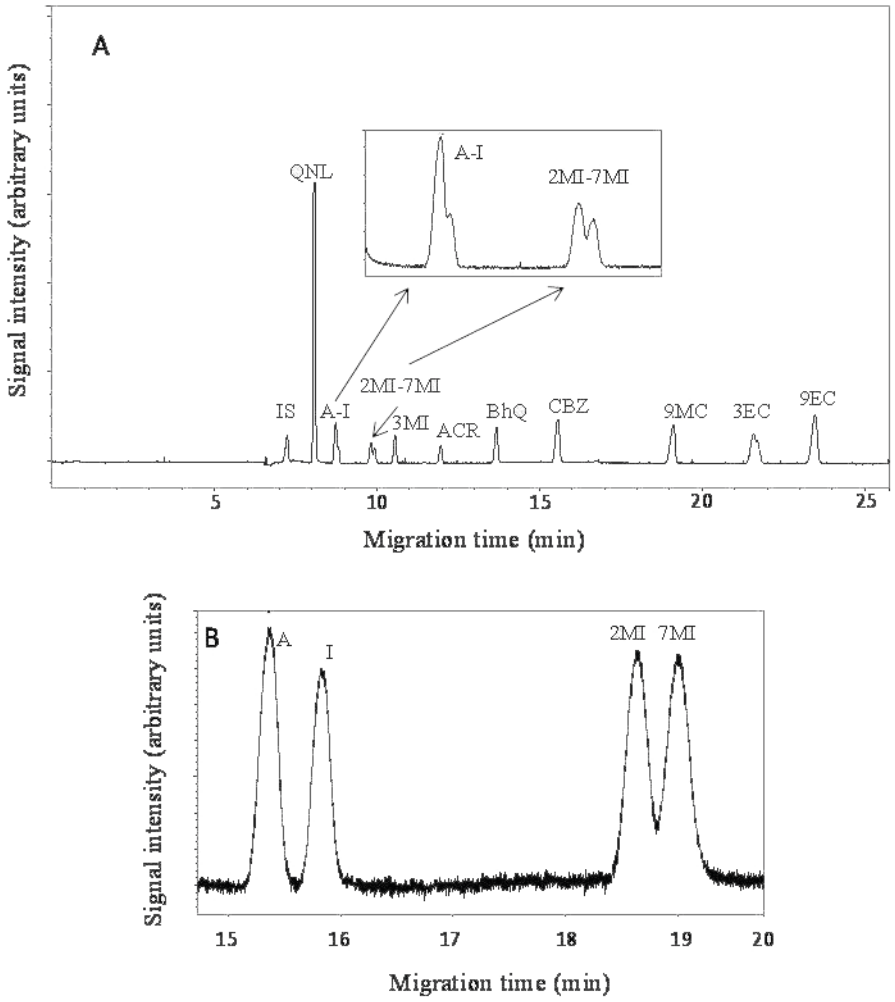


Figure 4. A) MEKC electropherogram of quinoline (QNL), N,N-dimethylaniline (A), indole (I), 2-methylindole (2MI), 7-methylindole (7MI), 3-methylindole (3MI), acridine (ACR), Benzo[h]quinoline (BhQ), carbazole (CBZ), 9-methylcarbazole (9MC), 3-ethylindole (3EC), 9-ethylcarbazole (9EC) using pyrrole as internal standard (IS), sample introduction for 10 s at 50 mbar, applied voltage of 15 kV at 20 ºC, detection at 230 nm and BGE consisting of borate buffer (10 mmol L^{-1}; pH 9.5), SDS (30 mmol L^{-1}) acetonitrile (20%) and urea 2 mol L^{-1}). B) Application of 10 kV at 15 ºC to improve resolution of A-I and 2MI-7MI.

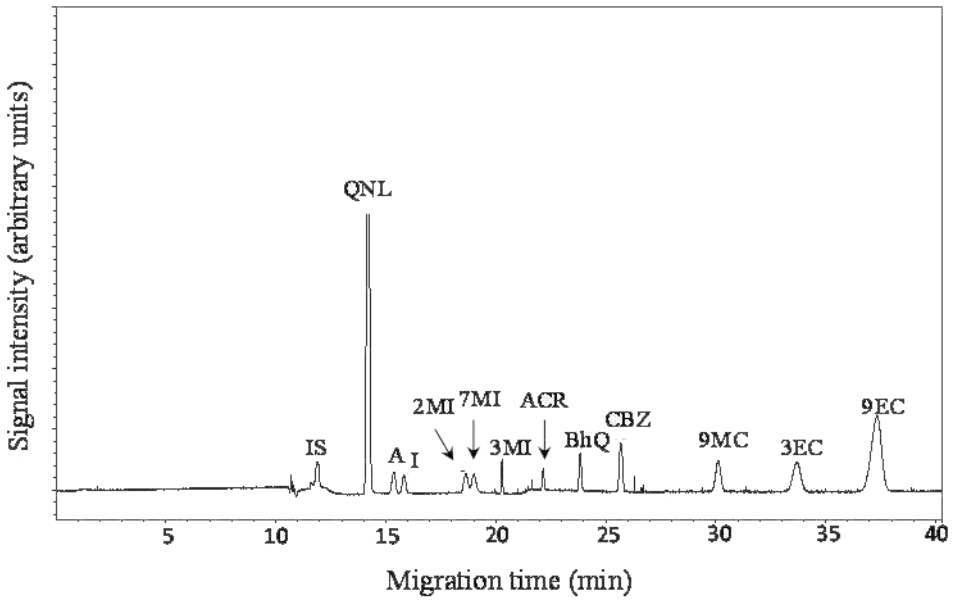


Figure 5. MEKC electropherogram of quinoline (QNL), N,N-dimethylaniline (A), indole (I), 2-methylindole (2MI), 7-methylindole (7MI), 3-methylindole (3MI), acridine (ACR), Benzo[h]quinoline (BhQ), carbazole (CBZ), 9-methylcarbazole (9MC), 3-ethylindole (3EC), 9-ethylcarbazole (9EC) using pyrrole as internal standard (IS), sample introduction for 10 s at 50 mbar, at 15 ºC, detection at 230 nm and BGE consisting of borate buffer (10 mmol L^{-1}; pH 9.5), SDS (30 mmol L^{-1}) acetonitrile (20%) and urea 2 mol L^{-1}) applied voltage of 10 kV from 0 to 20 min and 30 kV from 20 to 40 min.

Table I. Chosen conditions to separation and quantification of 12 NCACs using MECK

Parameter	Condition
BGE	10 mmol L^{-1} H_3BO_3/SDS 30 mmol L^{-1}
Organic modifier	Acetonitrile (20% in volume) and urea 2 mol L^{-1}
pH	9.5
Applied voltage gradient	5 kV (0 – 20 min) and 30 kV (20 – 40 min)
Temperature	15 ºC
Pressure of sample intoduction	50 mbar
Sample introduction time	10 s
Capillary lenghts: efective/total	47/55 cm
Internal diameter	50 µm
Detection (absorbance)	230 nm
Solution to prepare the analysis microemulsion	Ethanol (25%)/BGE (75%)

Table II. NCACs peak characteristics and resolution

NCACs[a]	Migration time (min)	Peak width (min)	Peak simmetry	Resolution
N-methylpyrrole (P)	11.9	0.15	1.12	baseline
Quinoline (QNL)	14.2	0.15	1.01	baseline
N,N-dimethylaniline (A)	15.4	0.13	1.28	baseline
Indole (I)	15.8	0.13	1.02	baseline
2-methylindole (2MI)	18.6	0.16	1.00	2,31[b]
7-methylindole (7MI)	19.0	0.17	1.35	baseline
3-methylindole (3MI)	20.3	0.07	1.05	baseline
Acridine (ACR)	22.2	0.07	1.00	baseline
Benzo[h]quinoline (BhQ)	23.8	0,10	0.87	baseline
Carbazole (CBZ)	25.7	0.13	0.82	baseline
9-methylcarbazole (9MC)	30.1	0.20	1.00	baseline
3-ethylcarbazole (3EC)	33.7	0.30	1.18	baseline
9-ethylcarbazole (9EC)	37.3	0.38	1.18	baseline

[a]NCACs = Nitrogen-containing aromatic compounds.

[b]Resolution (R_s) calculated using: $R_s = 2 \times (t_{m(2MI)} - t_{m(7MI)})/(w_{2MI} + w_{7MI})$ where t_m is migration time and w is peak width.

Analytical Parameters

Instrumental limit of detection (LOD) and limit of quantification (LOQ) were calculated by decreasing the concentration of each of the NCACs until reaching the lowest value that the software was able to perform peak integration. Such concentrations were introduced (in replicates) into the system and interpolated in the respective analytical curves (seven replicates) using six concentration points with equations shown in Table III. Those limits (Table III) were then assumed to be $x_m + 3s$ (for LOD) and $x_m + 10s$ (for LOQ) with x_m as the average recovered concentration in the curve and s the standard deviation of the seven replicates.

The repeatability (measured as coefficient of variation or CV) of the method was evaluated at three different concentrations (established for each analyte according to the sensibility of the analytical response) for each of the analytes using five consecutive analyzes of microemulsion containing analyte standards. Best results were achieved for QNL (CV up to 4%) and the poorer precision was for 9MC (CV of 11% in all concentrations) as seen in Table IV. These isooctane microemulsions were stored for 24 h before repeating the analysis again (repeatability) and, for all of the analytes, the results were statistically similar to the ones achieved in the previous day (two-tail Student *t*-test with 95% confidence level and n=7).

Table III. Analytical figures of merit to separation and quantification of 12 NCACs using MECK

NCACs[a]	Linear equation[b]	LOD (mg L^{-1})	LOQ (mg L^{-1})
Quinoline (QNL)	y = 0.74x + 0.13	0.78	1.03
N,N-dimethylaniline (A)	y = 0.91x + 0.066	4.86	7.96
Indole (I)	y = 0.32x - 0.087	1.09	1.99
2-methylindole (2MI)	y = 0.35x - 0.027	1.11	2.10
7-methylindole (7MI)	y = 0.30x - 0.063	1.09	1.56
3-methylindole (3MI)	y = 0.32x + 0.15	0.79	1.34
Acridine (ACR)	y = 0.059x + 0.078	3.98	4.62
Benzo[h]quinoline (BhQ)	y = 0.16x + 0.013	1.61	2.26
Carbazole (CBZ)	y = 0.21x + 0.029	1.08	2.31
9-methylcarbazole (9MC)	y = 0.19x + 0.11	0.68	1.15
3-ehtylcarbazole (3EC)	y = 0.19x + 0.13	0.76	1.14
9-ethylcarbazole (9EC)	y = 0.33x - 0.31	0.67	1.53

[a]NCACs = Nitrogen-containing aromatic compounds.
[b]On equations y = intensity of analytical response, x = concentration of analyte.

Table IV. Repeatability values

NCACs[a]	Concentration (mg L^{-1})	Standard-deviation (mg L^{-1})	Coeficient of variation (%)
N,N-dimethylaniline (A)	24	1.7	7
	85	8.7	10
Indole (I)	2	0.05	3
	11	1.2	11
2-methylindole (2MI)	3	0.05	2
	12	0.2	2
7-methylindole (7MI)	1	0.06	6
	12	0.6	5
3-methylindole (3MI)	3	0.1	7
	12	0.1	5
Acridine (ACR)	9	0.9	10
	16	1.4	9
Benzo[h]quinoline (BhQ)	3	0.1	5
	16	0.9	3

Table IV. Repeatability values (Cont.)

NCACs[a]	Concentration (mg L^{-1})	Standard-deviation (mg L^{-1})	Coeficient of variation (%)
Carbazole (CBZ)	3	0.1	3
	15	1.5	10
9-methylcarbazole (9MC)	2	0.2	10
	11	1.2	11
3-ethylcarbazole (3EC)	2	0.1	5
	14	0.7	5
9-ethylcarbazole (9EC)	2	0.04	2
	16	1.4	9
Quinoline (QNL)	1	0.03	3
	12	0.5	4

[a]NCACs = Nitrogen-containing aromatic compounds

A specific interference study was made only for the pair 2MI and 7MI, by varying their concentrations, from 3 to 30 mg L^{-1} in mixtures, in order to get a relative concentration proportion between them of 1 to 10 to 10 to 1. Enough resolution was achieved in these extreme proportions (R_s of 1.6 for 2MI:7MI at 1:10 proportion and R_s of 1.8 for 2MI:7MI at 10:1 proportion) which guarantee enough resolution to selectively determine these similar indole derivatives.

Finally, an interference test was made by fortifying a diesel sample with a specific concentration of each analyte and performing a recovery test. The diesel sample used was the one that presented the lowest amount of the NCACs (evaluated by the HPLC method reported by da Cunha *et al.* [29]). The electropherogram of the non-fortified diesel sample was used as blank. The recovered results (Table V) varied between 91 ± 7% (3EC) to 106 ± 4% (CBZ) indicating a good selectivity towards other components present in the diesel matrix. Another set of NCACs fortified diesel sample was analyzed by the proposed MEKC method and by using the HPLC method used to evaluate the baseline concentrations of NCACs at mg L^{-1} level. In this study, P, 2MI and 7MI were not included since the protocol reported for the HPLC did not include these three NCACs. The results for the quantified analytes were similar as indicated by a two-tailed Student *t*-test (with 95% confidence level and n=3). Therefore, the proposed method provides reliable information on quantification of NCACs.

Table V. Recovery of NCACs in diesel fortified samples

NCACs[a]	Fortification (mg L^{-1})	Recovered concentration (mg L^{-1})	Recovery (%)
Quinoline (QNL)	11.6	11.4 ± 0.1	98 ± 1.2
N,N-dimethylaniline (A)	10.9	9.3 ± 0.5	85 ± 4.3
Indole (I)	4.7	4.8 ± 0.2	102 ± 4.2
2-methylindole (2MI)	5.2	5.4 ± 0.9	103 ± 5.0
7-methylindole (7MI)	5.2	4.7 ± 0.01	90 ± 0.2
3-methylindole (3MI)	5.2	4.8 ± 0.2	92 ± 3.7

Table V. Recovery of NCACs in diesel fortified samples (Cont.)

NCACs[a]	Fortification (mg L^{-1})	Recovered concentration (mg L^{-1})	Recovery (%)
Acridine (ACR)	16.1	16.3 ± 1.2	101 ± 7.7
Benzo[h]quinoline (BhQ)	7.2	7.1 ± 0.5	99 ± 7.1
Carbazole (CBZ)	6.7	7,1 ± 0.2	106 ± 3.5
9-methylcarbazole (9MC)	7.2	7,3 ± 0.5	102 ± 7.0
3-ethylcarbazole (3EC)	7.8	7.1 ± 0.5	91 ± 6.8
9-ethylcarbazole (9EC)	7.8	7.5 ± 0.2	96 ± 2.2

[a]NCACs = Nitrogen-containing aromatic compounds

Analysis of real samples

Samples (diesel and a mix of diesel and gasoil) were provided by Petrobras. Aliquots of these were diluted in isooctane to be prepared as detergentless microemulsions before final dilution with BGE to form the analysis microemulsion to be introduced into the capillary. Sample amounts dissolved in isooctane varied in function of the sample characteristics (viscosity and density) and after a final volume adjustment, to produce a stable introduction microemulsion, the dilution factors varied from 4.000 to 12.000.

Despite the high dilution factor, the presence of NCACs was detected in most samples but in two of them (named I and II), a number of NCACs could be effectively determined. A prior elemental analysis has indicated a total N content of 0.4% in sample I (86.6% of C and 10.2% of H) and of 0.3% in sample II (89.0% of C and 9.2% of H). The N content comprises all of nitrogen compounds present in samples with a fraction being NCACs.

In sample I, the analytes 9EC and 9MC were found respectively at 6.5 ± 0.2 g L^{-1} and 2.4 ± 0.3 g L^{-1} (values in the original sample after correcting for sample dilution). In Figure 6 the electropherogram of sample I fortified with some NCACs (electropherogram A) and the one of the original sample (electropherogram B).

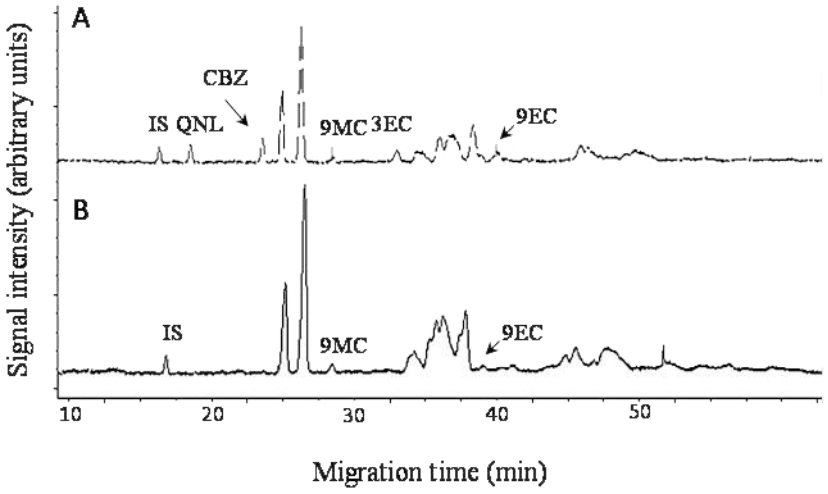


Figure 6. MEKC electropherogram of sample I with sample introduction for 10 s at 50 mbar, at 15 ºC, detection at 230 nm and BGE consisting of borate buffer (10 mmol L^{-1}; pH 9.5), SDS (30 mmol L^{-1}) acetonitrile (20%) and urea 2 mol L^{-1}) applied voltage of 10 kV from 0 to 20 mim and 30 kV from 20 to 40 min. A) fortified with some NCACs; B) non-fortified sample.

For sample II, concentrations (considering the correction of the dilution factor) of QNL (at 10.8 ± 0.7 g L^{-1}) and 3EC (at 11.8 ± 0.8 g L^{-1}) were found as can be seen in Figure 7. It is important to point out the presence of many unidentified absorption peaks maybe comprising a group of aromatic compounds not included in this work.

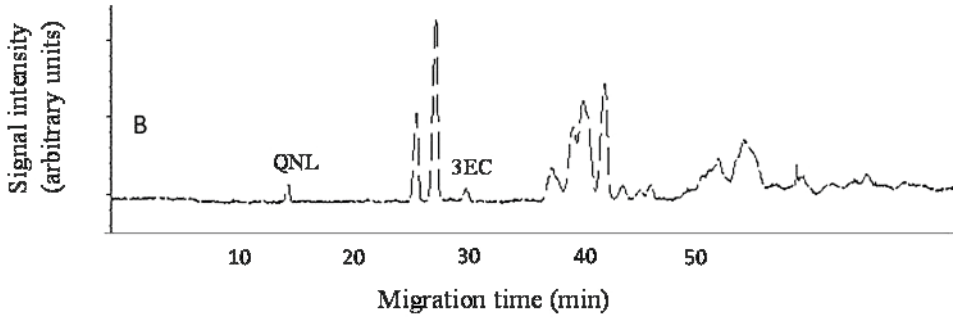


Figure 7. MEKC electropherogram of sample II (non-fortified sample) with sample introduction for 10 s at 50 mbar, at 15 ºC, detection at 230 nm and BGE consisting of borate buffer (10 mmol L^{-1}; pH 9.5), SDS (30 mmol L^{-1}) acetonitrile (20%) and urea 2 mol L^{-1}) applied voltage of 10 kV from 0 to 20 mim and 30 kV from 20 to 40 min.

CONCLUSIONS

In this exploratory study, the feasibility of separation of NCACs in diesel by MEKC without any sample treatment is demonstrated. The compatibilization of the oily sample and the aqueous BGE was achieved by using microemulsions. The high sample dilution factor was crucial to enable sample stabilization during electrophoretic separation but affected the capability for the detection of NCACs in real samples using absorption photometry. Despite that, NCACs could be detected in many of the samples and quantified in two of them. However, the use of laser-induced fluorescence, even exciting in a wavelength detuned from the maximum excitation, will certainly improve detection capability and also, in some extent, the selectivity because of the choice of excitation/emission pair. When analyzing real samples, migration times of the NCACs may be affected due to the complexity of the matrices, even in diluted conditions, therefore, the use of the internal standard and the NCACs standard addition are important to make the identification of the analytes.

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REFERENCES

1. Snyder, L. R.; Buell, B. E. *Anal. Chem.* **1968**, *40,* pp 1295-1302 (http://dx.doi.org/10.1021/ac60264a004).
2. Snyder, L. R. *Acc. Chem. Res.* **1970**, *3*, pp 290-299 (http://dx.doi.org/10.1021/ar50033a002).

3. Snyder, L. R; Buell, B. E. *J. Chem. Eng. Data*. **1966**, *11* (4), pp 545-553 (http://dx.doi.org/10.1021/je60031a022).
4. Speight, J. G. *The chemistry and technology of petroleum*, CRC Press, Fourth Edition, **2006**.
5. Bezinger, N. N.; Abdurakhmanov M. A.; Gal'pern G. *Pet. Chem. USSR.* **1962**, *1*, pp 13-19 (http://dx.doi.org/10.1016/0031-6458(62)90048-5).
6. Chawla, B. *J. Chromatography. Sci.* **1997,** *3*, pp 97-104 (http://dx.doi.org/10.1093/chromsci/35.3.97).
7. Okumura, L. L.; Stradiotto, N. R. *Talanta* **2007**, *72*, pp 1106-1113 (http://dx.doi.org/10.1016/j.talanta.2007.01.001).
8. Okumura, L. L.; Stradiotto, N. R. *Electroanal.* **2007**, *19*, pp 709-716 (http://dx.doi.org/10.1002/elan.200603804).
9. Cheng, X.; Zhao, T.; Fu, X.; Hu, Z. *Fuel Proc. Technol.* **2004**, *85*, pp 1463-1472 (http://dx.doi.org/10.1016/j.fuproc.2003.10.004).
10. Laredo, G. C.; Leyva, S.; Alvarez, R.; Mares, M. T.; Castillo, J.; Cano, J. L. *Fuel* **2002**, *81*, pp 1341-1350 (http://dx.doi.org/10.1016/S0016-2361(02)00047-9).
11. Kamata, K.; Motohashi, N.; Meyer, R.; Yamamoto, Y. *J. Chromatogr. A.* **1992**, *596*, pp 233-239 (http://dx.doi.org/10.1016/0021-9673(92)85012-I).
12. Bauserman, J. W.; Nguyen, K. M.; Mushrush, G. W. *Petrol. Sci. Technol.* **2004**; *22*, pp 1491-1505 (http://dx.doi.org/10.1081/LFT-200027799)
13. Li, B.; Liang, Y.; Hu, Y.; Du, Y.; Song, Y.; Cui, H. *Talanta*, **2003**, *61*, pp 803-809 (http://dx.doi.org/10.1016/S0039-9140(03)00383-7).
14. Adam, F.; Bertoncini, F.; Dartiguelongue, C.; Marchand, K.; Thiebaut, D.; Hennion, M. C., *Fuel*, **2009**, *88*, pp 938-946 (http://dx.doi.org/10.1016/j.fuel.2008.11.032).
15. von Muhlen, C.; Oliveira, E. C.; Morrison, P. D.; Zini, C. A.; Caramão, E. B.; Marriot, P. J. *J. Sep. Sci.* **2007**, *18*, pp 3223-3232 (http://dx.doi.org/10.1002/jssc.200700172).
16. Wang F. C.; Robbins, W. K.; Greaney, M. A. *J. Sep. Sci.* **2004**, *27*, pp 468-472 (http://dx.doi.org/10.1002/jssc.200301643).
17. von Muhlen, C.; Oliveira, E. C.; Morrison, P. D.; Zini, C. A.; Caramão, E. B.; Marriot, P. J. *Energy Fuels* **2010**, *24*, pp 3572-3580 (http://dx.doi.org/10.1021/ef1002364).
18. Oliveira, E. C.; Campos, M. C. V.; Lopes, A. S.; Vale, M. G. R.; Caramão, E. B. *J. Chromatogr. A*. **2004**, *(1-2)*, pp 171-177 (http://dx.doi.org/10.1016/j.chroma.2003.08.088).
19. Dijkmans, T.; Djokic, M. R.; Van Geem, K. M.; Marin, G. B. *Fuel* **2015**, *140*, pp 398-406 (http://dx.doi.org/10.1016/j.fuel.2014.09.055).
20. Terra, L. A.; Filgueiras, P. R.; Tose, L. V.; Poppi, R. J. *Fuel* **2015**, *160*, pp 274-281 (http://dx.doi.org/10.1016/j.fuel.2015.07.099).
21. Mao, J.; Pacheco, C. R.; Traficante, D. D.; Rosen, W. *J. Chromatogr. A*. **1994**, *684*, pp 103-111 (http://dx.doi.org/10.1016/S0021-9673(94)89136-2).
22. Mao, J.; Pacheco, C. R.; Traficante, D. D.; Rosen, W. *J. Liq. Chromatogr.* **1995**, *18*, pp 903-916 (http://dx.doi.org/10.1080/10826079508010401).
23. Mao, J.; Pacheco, C. R.; Traficante, D. D.; Rosen, W. *Fuel*, **1995**, *74*, pp 880-887 (http://dx.doi.org/10.1016/0016-2361(95)93942-7).
24. Luz, E. R.; Gonsalves, F. M.; Aucelio, R. Q. *J. Sep. Sci.* **2009**, *32*, pp 2058-2065 (http://dx.doi.org/10.1002/jssc.200900040).
25. Nascimento, P. C.; Gobo, L. A.; Bohrer, D.; Carvalho, L. M.; Cravo, M. C.; Leite, L. F. M. *J. Sep. Sci.* **2015**, *38*, pp 4055-4062 (http://dx.doi.org/10.1002/jssc.201500893).
26. Luz, E. R.; de Souza, C. F.; Sá, A.; da Cunha, A. L. M. C.; Franco, C. F. J.; Mello, S. C.; Aucélio, R. Q. *Fuel* **2014**, *129*, pp 20-26 (http://dx.doi.org/10.1016/j.fuel.2014.03.012).
27. Garguilo, M. G.; Thomas, D. H.; Anex, D. S.; Rakestraw, D. J. *J. Chromatogr. A.* **2000**, *883*, pp 231-248 (http://dx.doi.org/10.1016/S0021-9673(00)00409-X).

28. Fomin, A. N.; Smirnova, A. V.; Semenov, M. B.; Smirnova, E. V. *Pharm. Chem. J.* **2010**, *44*, pp 514-516 (http://dx.doi.org/10.1007/s11094-010-0506-6).
29. da Cunha A. L. M. C.; Sá, A.; Mello, S. M.; Vásquez-Castro, Y. E.; Luna, A. S.; Aucelio, R. Q. *Fuel* **2016**, *176*, pp 119-129 (http://dx.doi.org/10.1016/j.fuel.2016.02.035).
30. Segura-Carretero, A.; Cruces-Blanco, C.; Canabate-Diaz, B.; Fernandez-Sanches, J. R.; Fernandez-Gutierrez, A. *Anal Chim. Acta* **2000**, *417*, 19-30 (http://dx.doi.org/10.1016/S0003-2670(00)00917-X).
31. de Souza, R. M.; Mathias, B. M.; Scarmínio, I. S.; da Silveira, C. L. P.; Aucélio, R. Q. *Microchim. Acta* **2006**, *153*, pp 219-225 (http://dx.doi.org/10.1007/s00604-005-0461-1).
32. Cardoso, C. E.; Pacheco, W. F.; Sarubi, R.; Ribeiro, M. L. N.; Farias, P. A. M.; Aucélio, R. Q. *Anal. Sci.* **2007**, *23*, pp 1065-1069 (http://dx.doi.org/10.2116/analsci.23.1065).
33. Remers, W. A.; Gibs, G. J.; Pidacks, C.; Weiss, M. J. *J. Org. Chem.* **1971**, *36*, pp 279-284 (http://dx.doi.org/10.1021/jo00801a009).
34. Fetzner, S. *Appl. Microbiol. Biotechnol.* **1998**, *49*, pp 237-250 (http://dx.doi.org/10.1007/s002530051).
35. Scypinski, S.; Love, L. J. C. *Anal. Chem.* **1984**, *56*, pp 331-336 (http://dx.doi.org/10.1021/ac00267a007).
36. Martin, R. B. *Proc. Natl Acad. Sci. U.S.A.* **1974**, *71*, pp 4346-4347.
37. Ulrich, K. J.; Rumrich, G.; Neiteler, K.; Fritzsch, G. *Eu. J. Physiol.* **1992**, *420*, pp 29-38 (http://dx.doi.org/10.1007/BF00378638).
38. [38] Zachara, J. M.; Ainsworth, C. C.; Cowan, C. E.; Thomas, B. L. *Environ. Sci. Technol.* **1987**, *21*, pp 397-402 (http://dx.doi.org/10.1021/es00158a010).