

Precision in Petroleomics via Ultrahigh Resolution Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

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ABSTRACT: The ultrahigh resolution and mass accuracy of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) with the aid of soft ionization techniques, such as electrospray ionization (ESI), have been shown to provide fast and comprehensive class composition information of crude petroleum samples and distillates. This information has been translated into the major geochemical properties of the oil, but the level of reliability of the petroleomic approach by FT-ICR MS to compare samples or to predict sample characteristics remains an open question. To access this fundamental figure of merit, we have studied the repeatability and reproducibility of the ESI FT-ICR MS petroleomic method by using representative samples analyzed by different analysts and laboratories. In addition, the mean compatibility of the relative ion abundance obtained by two similar 7.2 T instruments located in different laboratories was evaluated. The data were analyzed via statistics tools, such as analysis of variance (ANOVA) to evaluate the repeatability and reproducibility among days and analysts, paired Student's *t*-test to compare the means obtained from different laboratories among different typical samples, and control charts to monitor the analytical system. A high degree of precision in petroleomic studies by FT-ICR MS has been found, particularly for the most abundant classes of components and, hence, for the classes currently used for property evaluation.

INTRODUCTION

The ultrahigh resolution and mass accuracy of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) in association with electrospray ionization (ESI),¹ a soft spray-based ionization technique performed under atmospheric conditions, provide proper ionization with little or no fragmentation of the polar components of crude oil (CO). The FT-ICR MS technique, with its unsurpassed mass resolution and mass accuracy, enables a molecular-level analysis of complex petroleum mixtures on the basis of accurate mass measurements, when unique elemental compositions ($C_cH_hN_nO_oS_s$) can be generated. If the resolution and the mass accuracy are sufficiently high, the technique enables fast and direct molecular formula determination via a "one ion-one component" of a heteroatom class relationship of the many thousand components of the CO polar fraction. These polar components generally comprise no more than 5–15% of the CO but are believed to be the most ancient CO molecules that have therefore been found to provide reliable information about geochemical history and chemical properties.^{2–7,34}

Obtaining reproducible and reliable ESI FT-ICR MS data directly from the very diverse CO samples—with most complex chemical mixtures—and interpreting them in a way that leads to proper molecular class assignments involves many analytical challenges. The occurrence of large differences in chemical properties of the many different CO classes, ranging from apolar to highly polar, which display drastic differences in ESI ionization efficiencies, necessarily means that CO components will not be

quantitatively represented in the ESI MS data. These non-quantitative responses are mostly due to ionic suppression, whereas the lack of reference standards for such complex matrixes obstructs attempts to correct for such deviations.⁸

In addition, many variables, such as solvent, additives, and ESI conditions such as spray, voltage, and temperature parameters, significantly alter spectra distributions. Additionally, at ultrahigh resolution, a typical CO mass spectrum detects many thousands of ions with many peaks per nominal *m/z* value, in addition to isotopologue ions, posing a great challenge for detection, analysis, and interpretation.^{9,10}

A most fundamental question that arises when performing direct petroleomic analysis via ESI FT-ICR MS is related to its precision as measured by reproducibility and repeatability, as well as systematic error. Precision and systematic errors are indeed crucial because most FTMS petroleomic predictions are based on spectra comparison using relative ion abundances. Precision describes random error, whereas systematic error describes the tendency also known as bias, and the accuracy (closeness to the true value of a single measurement or a mean value) incorporates both types of errors.^{11,12} Precision (repeatability and reproducibility) and accuracy together determine the error of an individual determination and are the most important criteria for judging the performance of an analytical procedure. There is also

Received: April 11, 2013

Revised: October 21, 2013

Published: December 4, 2013



a compromise between precision and accuracy with speed and easiness of the determination, and the best balance between these two opposing parameters should be found by evaluating the sources of variation. Repeatability shows the variation when all the factors involved are the same, and reproducibility or intermediate precision (if measured in the same laboratory) show the variation when one or more factors are different (such as days, analysts, and equipment).³³

In petroleomic studies by ESI FT-ICR MS, the quality indicators and major parameters affecting precision are yet to be determined. Ionization efficiency should be crucial due to the highly complex synergic effects that make the ionization efficiency of molecules greatly affected by ion suppression due to the presence and relative concentration of a multitude of other species in the ESI droplet. For example, ESI in the positive ion mode favors the most basic components whose presence and relative concentration suppress the ionization of species of lower basicity.^{13–16} In addition, the mass accuracy in FT-ICR MS also depends on factors including the cell design, cell parameters used, magnetic field strength, ion population, and ion cloud behavior within the ICR cell,^{17–22} whereas the ion current may vary widely from scan to scan or the ionization efficiency may vary between samples. Peak shapes are also important because asymmetrical peaks may shift the center of mass, leading to lower accuracy.³⁵

In FT-ICR MS, the highest accuracies are obtained with small ion populations, with calls for the use of mass spectra averaging.²³ At higher ion populations, space charge effects can enhance mass errors, and at too low ion populations, the contribution of the noise ultimately becomes limiting and degrades the mass accuracy as well as the dynamic range.²⁴ For large dynamic ranges, however, large ion populations are desired, which cause relatively large space charge induced frequency shifts, therefore reducing the accuracy.²⁴

A major source of variations in ESI FT-ICR MS is the ion–ion interactions inside the ICR cell, but white noise (constant noise power distribution at all frequencies) caused by thermal effects could also be critical. Some of this noise can be reduced before the measurement, for example, by optimization of the ICR cell and acquisition parameters, or after the measurement during the preprocessing stages. Generally, the overall signal-to-noise ratio (SNR) in a mass spectrum can be improved by acquiring more scans: the magnitude of additive white Gaussian noise (AWGN) increases as $n^{1/2}$ (where n is the number of scans acquired) and the signals increase as a function of n . Therefore, the overall SNR is, in principle, proportional to $n^{1/2}$. In reality, the actual SNR observed is also affected by other non-white-noise effects, including instrument parameter drifts that can change the relative intensities of the peaks during the experiments, which can contribute to an increase in the variability of the measured intensities.^{25–27}

For the data treatment, the crucial step is that of discarding the noise while retaining and then measuring the real peaks. For a single spectrum, this is typically achieved by setting an arbitrary peak area threshold or an SNR threshold and retaining only peaks that exceed this specification. The SNR thresholds are usually 3:1 (the limit of detection) and 10:1 (limit of quantification).²⁵ The calculation of the SNR is sometimes unclear and inconsistent, but some authors, such as Payne,²⁵ have used SNR as the ratio between the heights of the signal peak by the standard deviation of the white Gaussian noise in a signal free region of the spectrum. A “hard” threshold technique such as this will either result in many low abundance ions not being detected

or many noise peaks being falsely counted as real. In addition, this strategy can also incorrectly remove ions with abundances that are intermittently reduced by adverse effects during ESI, such as ion suppression.²⁵

This paper has attempted to evaluate how precision affects petroleomic studies using FT-ICR MS and how reliable are the data obtained in different laboratories. For these purposes, a 7.2 T FT-ICR MS (Thermo Scientific Instruments) was used as a model case. We have also studied the method's precision and mean compatibility between the laboratories involved, considering the relative mass abundance given by the ionizable compounds present in the crude oil samples. We considered the main factors that can interfere, such as analysts, reproducibility on different days, the same equipment model operated in two different laboratories, and different CO samples. Typical CO samples were selected to guarantee, as much as possible, the representativeness of such a complex matrix. Although we believe that different FT-ICR cells working at similar resolution and accuracy should, in principle, display similar precision, the influence due to the use of different cells and magnetic field strengths has not been evaluated and remains an open question.

EXPERIMENTAL SECTION

Sample Preparation. The CO samples from different Brazilian oil fields were prepared by dissolving 2 mg of the crude sample in 2 mL of a toluene:methanol 1:1 mixture. For ESI (+), formic acid 0.01% was added to facilitate protonation of the basic nitrogen-containing compounds to yield $[M + H]^+$ ions. For ESI (–), an aqueous solution of 0.01% ammonium hydroxide was added to facilitate deprotonation of the more acidic components to form $[M - H]^-$ ions. HPLC-grade methanol, toluene, ammonium hydroxide, and formic acid were purchased from Merck SA (Rio de Janeiro, Brazil) and were used without further purification.

Mass Spectrometry. Ultrahigh resolution MS was performed in a hybrid 7.2 T Fourier transform ion cyclotron resonance mass spectrometer (ThermoScientific, Bremen, Germany) in electrospray ionization mode. A scan range of m/z 200–1000 was used, and 100 microscans³⁶ were collected in each run. The average resolving power (Rp) was 400 000 at m/z 400. Time-domain data (ICR signal or transient signal) was acquired for 700 ms. The automatic gain control (AGC) used was 8×10^5 . The MW distribution for each sample was first verified by LTQ analysis to ensure the similarity of the MW distribution

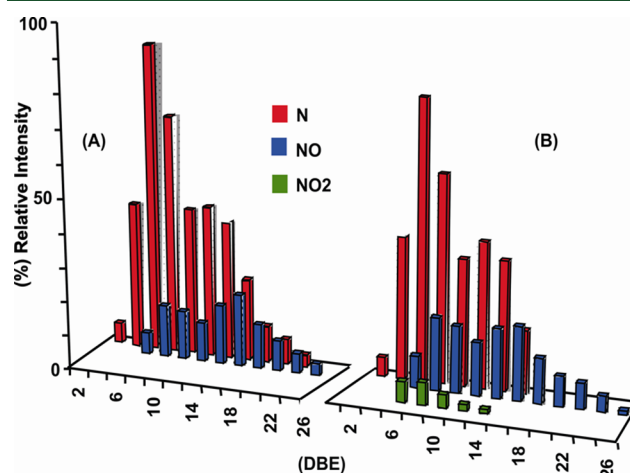


Figure 1. ESI (+) FT-ICR MS analysis based on the classes and DBE distribution using relative ion abundances for samples A and B (same lab, same analyst). Operational conditions: capillary voltage (3.10 kV), tube lens (148 V) –100 V negative mode, and flow rate of $5 \mu\text{L min}^{-1}$.

on the transfer to the FT-ICR MS cell where the number of ions should be kept as low as possible (8×10^5). The ESI conditions were capillary voltage (3.10 kV), tube lens (148 V) –100 V negative mode, and a flow rate of $5 \mu\text{L min}^{-1}$. Spectra were exported as .txt files containing the m/z values and the absolute relative intensities using the Xcalibur software 2.0 (ThermoScientific-Bremen, Germany) to be processed by the PetroMS 3.0 Beta program developed by Corilo.²⁸

Data Processing. Data processing was performed using the software Petro MS 3.0²⁸ developed at Thomson Laboratory, which enables the spectra recalibration based on homologous series distribution and allows the assignment of molecular formula, a graphic representation of the classes and the DBE distribution versus carbon number, also plotting the Kendrick's and Van Krevelen diagrams. Elemental formulas were calculated and assigned on the basis of the m/z values within a 1 ppm error range. The relative abundance of the individual peaks detected was grouped by classes and normalized to the summed relative abundance of each peak assigned. Elemental formulas were assigned and merged into a table, in which the information such as relative abundances for the ions found in each class was used for statistical analysis and sample comparison. Figure 1 illustrates, for two crude oil samples, how the ESI-FT-ICR data are grouped into classes and different DBE with the aid of the software treatment.

Quality indicators were established to determine if the spectra and the analytical system were suitable. The main quality indicators used were the following: (a) the spectra shape⁷ (Figure 2), which was adjusted to

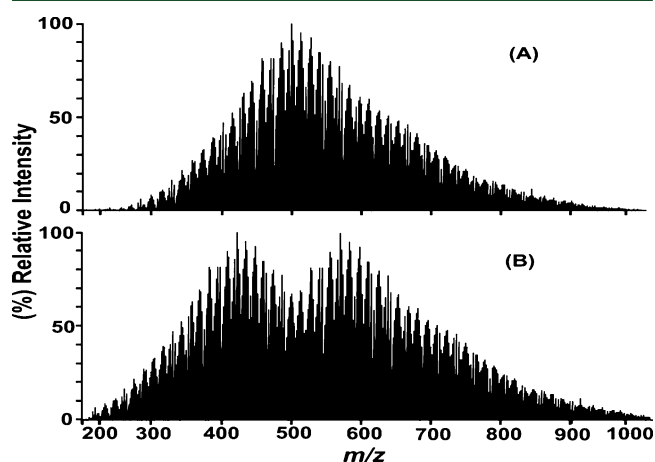


Figure 2. CO ESI (+) FT-ICR spectra shape judged by the symmetry of the Gaussian of ion distribution: (A) accepted shape; (B) not accepted shape.

be in the best Gaussian shape as possible with similar ion distributions, compared by visual inspection, to that obtained in the linear trap, and (b) the transient signal or FID (free induction decay) that should decay smoothly, showing that the number of ions in the ICR cell is optimal for most reduced space charge effects, as well as the best signal-to-noise ratios.^{7,25}

Table 2. Individual Results Obtained on Four Different Days by ESI (+) for Samples A and B

sample	N (%)	N ₂ (%)	NO (%)	NOS (%)
A_D1	57.70	12.40	11.00	22.60
A_D2	65.90	17.70	12.00	18.40
A_D3	57.90	16.30	12.40	17.60
A_D4	56.20	15.20	13.10	16.00
A_D5	57.10	14.90	10.50	15.20
A_D6	56.10	13.80	12.30	20.20
A_D7	54.20	12.90	14.60	24.80
A_D8	56.00	16.50	15.50	24.20
B_D1	69.80	10.10	20.20	ND ^a
B_D2	71.40	9.00	19.60	
B_D3	68.20	10.70	20.00	ND ^a
B_D4	66.20	8.40	18.30	
B_D5	70.00	10.70	19.30	ND ^a
B_D6	68.70	10.90	20.40	
B_D7	69.30	12.80	17.90	ND ^a
B_D8	62.90	17.90	19.60	

^aNot detected.

Statistical Treatment. Briefly, two representative CO samples (A and B) were analyzed on different days by different analysts in the same laboratory via both ESI (+) and ESI (–) to evaluate the intermediary precision of the FT-ICR MS measurements. The data obtained on the same day by the same analyst were used to evaluate the repeatability. The relative abundances obtained for each class were analyzed using analysis of variance (ANOVA), and the values of repeatability and reproducibility were obtained as described in ISO 5725.²⁹ Variations due to different samples and analysts were studied with three samples (coded as M, N, and O) and two different analysts in the same laboratory used the software Minitab version 7 to aid the data and graphics analysis. Excel spreadsheets (data analysis tool) were also used for ANOVA treatment. The data obtained were compared with critical values found from statistics tables; for example, the critical value for a two-tailed test with a significance level of 0.05 (confidence level of 95%) and five degrees of freedom would lead to a Student's *t* critical value of (0.05, 5).

Data compatibility between the two laboratories (UNICAMP and CENPES) was evaluated by comparing a set of 10 different CO samples for ESI (+) and eight samples for ESI (–) for their relative class abundances. The samples were carefully chosen to have as different physical chemical properties as possible, to guarantee representativeness in the crude oil sample world. Statistical treatment was performed using a paired *t*-test, as explained elsewhere.^{30,31} Graphics were obtained using Minitab software version 7. The level of significance established was 0.05, and two tails were considered. The statistics tool *t*-paired allowed comparison of the results from two different sets of samples analyzed by different treatments or, in our study, by different laboratories. The resulting *t* (stat *t*) obtained through the average of the differences for each pair of means divided by the difference standard deviation and multiplied by the square root of the number of observations, according

Table 1. Results of Precision on Different Days for Samples A and B

<i>n</i> = 8		positive mode				negative mode					
sample	value	N	N ₂	NO	NOS	N	NO	NO ₂	O	O ₂	OS ₃
A	REPE (%)	10.2	7.8	2.8	8.0	7.5	1.4	2.8	2.4	2.6	ND ^a
	REPRO (%)	15.5	5.9	6.0	12.9	10.5	2.8	6.0	8.2	4.4	
	CV (%)	5.3	15.6	6.6	12.0	2.2	1.9	6.6	4.5	3.5	
	MEAN (%)	57.6	15.0	12.7	19.9	23.7	22.2	12.7	16.2	22.1	
B	REPE (%)	8.3	6.8	3.2	ND ^a	4.6	2.6	13.3	1.6	1.6	7.4
	REPRO (%)	9.0	10.4	2.9		7.2	2.9	13.3	7.4	1.6	7.4
	CV (%)	3.6	17.8	4.9		4.2	4.9	76.5	2.2	1.9	75.2
	MEAN (%)	68.3	11.3	19.4		32.8	16.1	5.2	21.6	25.3	3.0

^aNot detected.

Table 3. Individual Results Obtained on Four Different Days by ESI (–) for Samples A and B

sample	N (%)	NO (%)	NO ₂ (%)	O (%)	O ₂ (%)	OS ₃ (%)
A_D	26.40	22.40	11.00	18.20	22.10	ND ^a
A_D	26.30	22.50	12.00	18.00	22.00	
A_D	26.10	22.40	12.40	15.70	23.40	ND ^a
A_D	20.10	21.60	13.10	14.00	22.10	
A_D	26.30	23.10	10.50	18.60	21.60	ND ^a
A_D	24.30	22.30	12.30	18.10	20.20	
A_D	20.30	21.30	14.60	13.00	23.20	ND ^a
A_D	20.10	21.60	15.50	14.00	22.10	
B_D1	35.70	16.40	3.60	22.70	25.10	4.70
B_D2	35.00	16.60	12.00	22.00	25.60	1.00
B_D3	35.00	16.60	5.00	22.80	25.60	2.00
B_D4	31.50	16.80	3.00	22.90	24.60	5.00
B_D5	31.40	17.20	8.00	23.30	24.60	3.00
B_D6	31.10	15.00	2.00	22.30	25.30	2.00
B_D7	30.60	15.30	2.00	18.60	25.60	1.00
B_D8	32.10	15.10	6.00	18.00	25.80	5.00

^aNot detected.

to eq 1, should be compared with the critical t . If the resulted t is lower than the critical, the differences among the laboratory means are not significant, and if it is greater, the differences are considered of significance within the 95% confidence interval.

$$t_{\text{calc}} = \frac{|\bar{d}| \sqrt{n}}{s_d} \quad (1)$$

where \bar{d} = mean of the differences between the pairs, s_d = standard deviation for the differences, and n = number of observations.

System suitability was monitored using control charts,³² which provides a tool to identify when the variation increases or when drift or bias occurs. A reference CO sample was analyzed at the beginning of

every working day to observe trends and to monitor the performance of the measurement system over time. To build the control chart, as explained elsewhere,³¹ a central line representing the arithmetic mean of at least the first 18 results is plotted on the chart. To obtain the warning (2σ) and the action limits (3σ), the control lines (set at two or three standard deviations) are plotted for the upper limit and for the lower limit.

For a normal distribution, the calculated warning limits correspond approximately to the 95% confidence level (two standard deviations) and the action limits correspond approximately to the 99.7% confidence level (three standard deviations). The results outside the action limits are, therefore, very unlikely to occur by chance, whereas results outside the warning limits are expected to occur, on average, about once per 20 observations.

If a result falls outside the action limits, the measurement process should be stopped and an investigation into the cause of the problem conducted. A laboratory should also have defined procedures defining proper actions if results fall between the warning limits and the action limits.

RESULTS AND DISCUSSION

Repeatability and Intermediate Precision. The precision of the method was evaluated by the two laboratories involved (ThoMSON Mass Spectrometry Laboratory - UNICAMP and Mass Spectrometry Laboratory of CENPES - PETROBRAS) via analysis of typical samples on different days, after all quality indicators and criteria of acceptance described in Data Processing had been achieved, as measured by spectra with a Gaussian shape and a FID (free induction decay) showing a soft decay versus time. Table 1 shows the summary of the statistical results obtained for two A and B samples analyzed twice on 4 different days via both ESI (+) and ESI (–) FT-ICR MS. The highest coefficients of variation in ESI (+) were 15.6% (sample A) and 17.8% (sample B) for the N₂ class, and the lowest were 5.3% (sample A) and 3.6% (sample B) for the N class. Considering

Table 4. Analysis of Variance (ANOVA) for ESI (+) for Samples A and B^a

source	N				NO				N ₂				F critical
	SQ	DF	MQ	F	SQ	DF	MQ	F	SQ	DF	MQ	F	
sample	237.52	2	118.76	36.34	70.95	2	35.47	8.169	69.42	2	34.71	25.64	3.885
columns	2.275	1	2.275	0.6964	39.60	1	39.60	9.111	16.05	1	16.05	11.86	4.747
interactions	21.87	2	10.93	3.347	3.763	2	1.881	0.432	15.86	2	7.933	5.862	3.885
within	39.20	12	3.267		52.11	12	4.342		16.24	12	1.353		
total variation	3300.87	117			1166.4	117			1177.5	117			

^aSQ = sum of squares. DF = degrees of freedom. MQ = mean square.**Table 5. Analysis of Variance (ANOVA) for ESI (–) for Samples A and B**

source	N				NO				NO ₂				F critical
	SQ	DF	MQ	F	SQ	DF	MQ	F	SQ	DF	MQ	F	
sample	2103.9	2	1051.9	24.82	387.7	2	193.8	151.5	84.73	2	42.36	4.011	3.885
columns	107.55	1	107.55	2.537	0.4355	1	0.4355	0.3404	4.013	1	4.013	0.3800	4.747
interactions	39.94	2	19.97	0.4713	4.301	2	2.150	1.680	0.1944	2	0.09722	0.009206	3.885
within	508.5	12	42.37		15.35	12	1.279		126.7	12	10.56		
total variation	2759.9	17			407.8	17			215.6	17			

source	O				O ₂				O ₃ S				F critical
	SQ	DF	MQ	F	SQ	DF	MQ	F	SQ	DF	MQ	F	
sample	1054.0	2	527.0	112.7	7357.7	2	3678.8	52.73	4.663	2	2.331	0.8981	3.885
columns	17.40	1	17.40	3.724	237.6	1	237.6	34.06	3.125	1	3.125	1.203	4.747
interactions	8.943	2	4.471	0.9568	64.11	2	32.05	0.4595	4.663	2	2.331	0.8981	3.885
within	56.08	12	4.673		837.1	12	69.75		31.15	12	2.596		
total variation	1136.4	17			8496.6	17			43.60	17			

Table 6. ESI (+) Data Comparing Two Different Analysts and Three Different Samples (M, N, O)

sample	analyst	N (%)	NO (%)	N ₂ (%)
M	1	91.6	3.2	3.6
		90.2	5.4	1.6
		90.5	2.1	3.0
	2	92.5	1.6	5.9
		91.6	2.0	5.5
		91.8	1.7	6.5
mean		91.4 ± 1.0	2.7 ± 1.7	4.4 ± 2.2
N	1	83.6	7.9	3.9
		84.3	4.1	6.3
		82.4	7.9	3.9
	2	87.4	2.8	5.8
		83.7	3.7	9.5
		88.6	1.3	8.4
mean		85.0 ± 2.8	4.6 ± 3.1	6.3 ± 2.6
O	1	85.8	7.8	2.2
		81.4	10.6	1.7
		84.5	8.7	1.8
	2	84.1	7.4	1.2
		81.9	8.9	0.0
		79.1	1.6	2.2
mean		82.8 ± 2.8	7.5 ± 3.6	1.5 ± 1.0
categories		2	1	1

classes with over 5% relative mass abundance, ESI (−) provided lower coefficients of variation. The highest values obtained were 6.6% for the NO₂ class (sample A) and 4.9% for the NO class (sample B). The lowest values were 1.9% for the NO class (sample A) and 1.9% for the O₂ class (sample B). Nevertheless, classes with under 5% relative mass abundance (NO₂, OS₃) might show high coefficients of variance (76.5, 75.2%).

Knowledge of the limits of repeatability and reproducibility, calculated as explained elsewhere,^{29,33} is also useful in understanding

the method's capability because this information can be used to establish internal criteria to accept or not accept differences among replicates (repeatability) and differences among other factors under study (days or analysts), called reproducibility or intermediary precision if done in the same laboratory. For example, the repeatability data (Table 1) shows that difference could be expected until 10% for replicates of the N class and 15% among days for ESI (+) and 7.5% for repeatability and 10.5% for reproducibility for ESI (−). In addition, reproducibility can also provide information of whether the method is able to measure a given process because you know the tolerance of the process or product specifications. Usually, a method's reproducibility should not be greater than 30% of the tolerance range.^{31,33}

Tables 2 and 3 show the individual results used for the statistical treatment.

The contribution of different analysts and the possible interactions among analysts and samples were studied by running a two-factor ANOVA with replication for all the classes found via ESI (+) and ESI (−).

ANOVA measures the amount of variability of the system itself and compares it to the total variability observed to determine the viability of the measurement system. Table 4 shows that the variability of the study is mostly due to the difference among the parts (samples), as the *F* calculated for the N class (36.3496), NO class (8.1692465), and N₂ class (25.648194) are greater than the critical *F* (3.8852938). As expected for classes with lower abundances, there might be differences and bias due to interactions between the sample and analyst, as shown by the calculated *F*, which is greater than the critical *F* for the N₂ class when using ESI (+).

For the classes detected by ESI (−), only variations due to the differences among the samples are detected (Table 5). ESI (−) is more discriminative and more suitable to discern among samples.

The capacity of the method to discern small differences among the samples was also tested. This capacity is calculated by the number of distinct categories, obtained by dividing the standard

Table 7. ESI (−) Results Comparing Two Different Analysts and Three Different Samples (M, N, O)^a

sample	analyst	N (%)	NO (%)	NO ₂ (%)	O (%)	O ₂ (%)	O ₃ S (%)
M	1	21.2	ND ^b	14.1	ND ^b	64.7	ND ^b
		29.3		5.8		64.9	
		27.5		4.8		67.7	
	2	35.6	ND ^b	9.0	ND ^b	55.4	ND ^b
		32.7		9.9		57.4	
		32.3		4.0		63.7	
mean and uncertainty		29.8 ± 5.8	ND ^b	7.9 ± 4.4	ND ^b	62.3 ± 5.5	ND ^b
N	1	45.5	4.2	7.2	7.1	36	ND ^b
		56.1	5.2	4.6	10.1	24.1	
		45.3	4.0	7.1	7.0	36.6	
	2	44.1	5.0	8.4	5.7	36.8	0.0
		38.3	3.3	5.2	4.6	48.5	0
		47.1	3.5	7.0	8.8	33.6	0.0
mean and uncertainty		46.0 ± 6.6	4.2 ± 0.9	6.6 ± 1.6	7.2 ± 2.3	35.9 ± 9.0	ND ^b
O	1	49.6	11.6	2.9	19.8	16.1	ND ^b
		52.1	10.3	2.5	21.3	13.8	
		46.5	10.5	3.0	18.3	21.6	
	2	51.6	11.7	2.5	18.9	15.3	0.0
		44.8	11.8	3.9	16.9	21.9	0.7
		46.9	11.0	0.0	15.6	26.5	0.0
mean and uncertainty		48.6 ± 3.4	11.2 ± 0.7	2.5 ± 1.5	18.5 ± 2.3	19.2 ± 5.6	ND ^b
categories		2	6	1	5	3	

^aUncertainty expressed in 95% confidence interval. ^bNot detected.

Table 8. Results Obtained by Two Different Laboratories for 10 Different Samples via ESI (+)

sample	N (%)		NO (%)		N ₂ O ₂ (%)	
	LAB1	LAB2	LAB1	LAB2	LAB1	LAB2
C	72.7	75.8	7.5	9.8	2.5	ND ^a
D	91.0	83.2	1.8	11.7	3.3	
E	80.2	83.6	10.0	10.6	0.0	
F	81.3	80.2	7.3	13.5	4.2	
G	81.3	80.2	12.2	16.2	0.0	
H	89.9	82.2	0.0	12.4	4.7	
I	82.2	77.4	11.6	19.9	4.0	
J	85.7	78.8	7.2	19.2	2.6	
K	66.0	58.2	15.0	21.2	4.2	
L	81.4	89.7	5.1	1.9	0.0	

^aNot detected.**Table 9. Results Obtained by Two Different Laboratories for Eight Different Samples via ESI (–)**

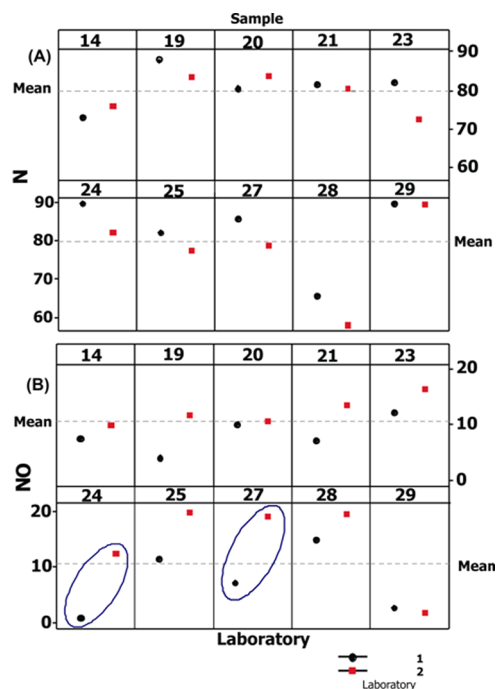
sample	N (%)		NO (%)		O (%)		O ₂ (%)	
	LAB1	LAB2	LAB1	LAB2	LAB1	LAB2	LAB1	LAB2
C	42.2	54.7	11.6	8.4	14.7	21.8	8.2	9.5
D	77.9	83.3	8.9	4.2	13.2	12.5	0.0	0.0
E	52.6	62.1	14.4	8.4	22.1	23.7	7.3	5.8
F	46.2	37.9	3.7	0.0	12.0	10.1	37.0	51.9
G	44.2	42.0	11.0	8.2	11.3	8.0	32.1	41.8
I	40.9	63.0	21.7	10.7	10.3	16.0	22.3	7.7
J	51.6	58.4	11.7	3.9	18.9	21.7	14.3	15.9
K	29.2	26.6	11.6	5.1	21.4	21.7	28.4	39.9

Table 10. *t*-Paired Test for ESI (+)

Result	N		NO	
	LAB1	LAB2	LAB1	LAB2
mean (%)	81.24	77.81	7.77	13.64
variance (%)	55.33	77.37	21.70	33.44
Pearson correl	0.6793		0.5514	
resulted <i>t</i>	1.631		–3.136	
critical <i>t</i>	2.262		2.261	

deviation for parts (samples) by the standard deviation for gage and then multiplying by 1.41.³²

The number of categories represents the number of non-overlapping confidence intervals that will span the range of product variation. It can also be understood as the number of groups within the processed data that the measurement system can discern. Tables 6 and 7 also show that samples can be better distinguished by ESI (–), where the number of categories is greater than 2 for the NO, O, and O₂ classes. When the number of categories is 2, the data can be divided into two groups: high

**Figure 3. Paired *t*-test for the two laboratories via ESI (+): (A) N class; (B) NO class. Circles emphasize the samples that show major differences.**

and low. When the number of categories is 3, the data can be divided into three groups: high, middle, and low.

Laboratory Comparison. A set of representative CO samples were analyzed via ESI (+) and ESI (–) FT-ICR MS by the two laboratories (Tables 8 and 9). The results were analyzed using the *t*-paired test stating the null hypothesis that the differences between the pairs are zero. Tables 10 and 11 show the statistical *t*-paired results and the Pearson coefficient of correlation, which measures the linear relationship between the variables. Note that the closer to 1, the higher the correlation between the variables. If the coefficient is negative, the relationship is inverse. The statistical analyses show that the resulted *t* (stat *t*) obtained for the N class is lower than the critical value, so the test is not significant at the chosen level of confidence, and the null hypothesis is accepted. However, if the resulted *t* obtained for the NO class is greater than the critical value, being significant at the chosen level of confidence, the null hypothesis must be rejected. Indeed, for ESI (–), except for the NO class, one can conclude that there is good agreement between the two laboratories (i.e., the resulted *t* is lower than critical *t*). That is, if one variable increases, the other decreases. The very high variance observed (Table 11) is expected because the samples are very different, to guarantee the representativeness of crude oil samples. Figures 3 and 4 show the results obtained for all samples for both laboratories, plotted to aid the comparison. A line representing the

Table 11. *t*-Paired for ESI (–)^a

result	N		NO		O		O ₂	
	LAB1	LAB2	LAB1	LAB2	LAB1	LAB2	LAB1	LAB2
mean	48.1	53.5	11.82	6.112	15.48	16.93	18.82	21.56
variance	197.6	310.9	25.64	11.82	21.82	37.41	174.6	392.7
Pearson correl	0.8363		0.8588		0.8077		0.9179	
resulted <i>t</i>	–1.575		5.878		–1.133		–0.83227	
critical <i>t</i> bi-tailed	2.364		2.364		2.364		2.364	

^aObservation number *n* = 8.

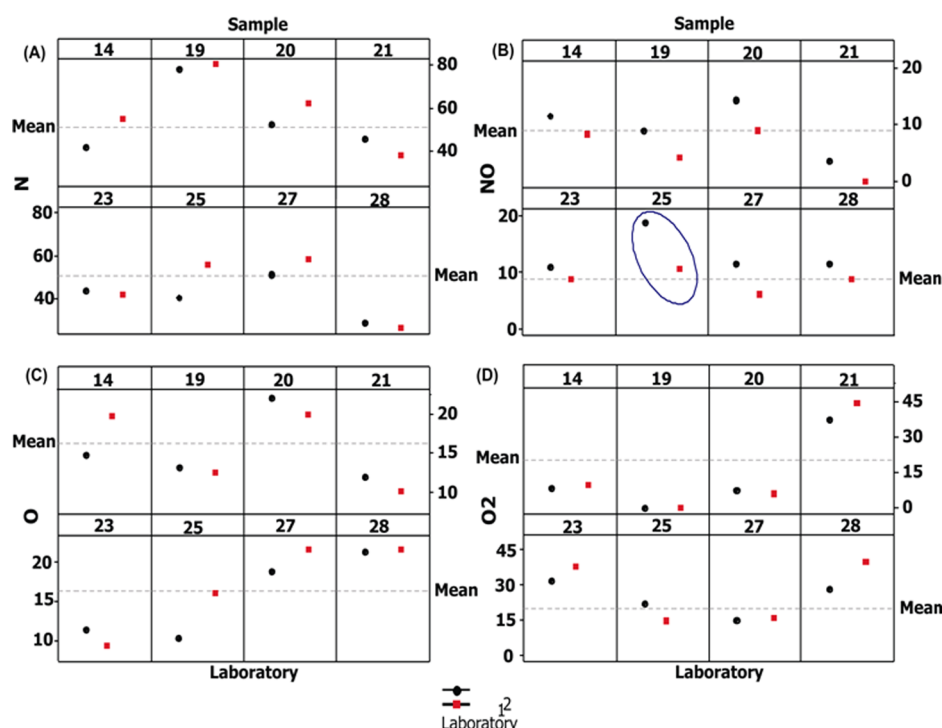


Figure 4. Paired t -test for the two laboratories via ESI (–): (A) N class; (B) NO class; (C) O class; (D) O₂ class. Circles emphasize the samples that show major differences.

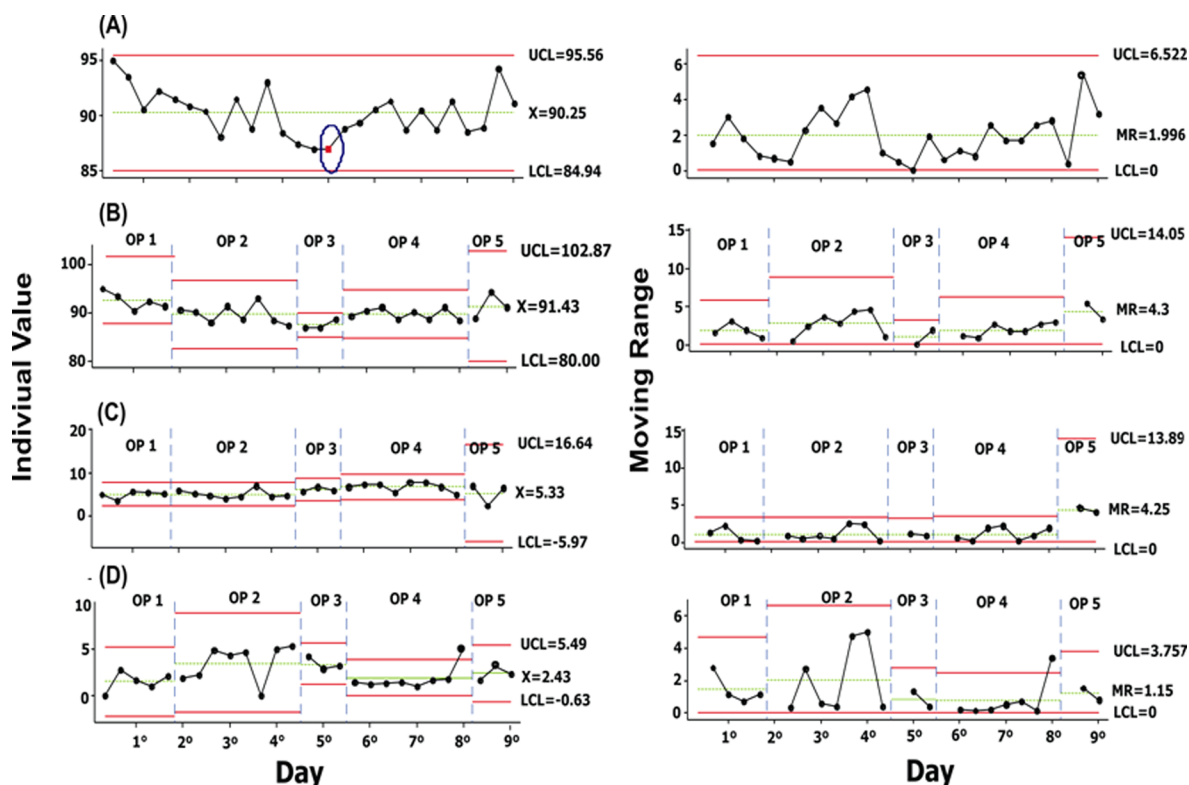


Figure 5. Control charts for individual values via ESI (+) and moving ranges: (A) N class; (B) N class by analyst; (C) N₂ class by analyst; (D) NO class by analyst.

mean for all the observations for each sample is plotted as a reference for comparison. The major differences are emphasized by circles.

Analytical System Monitoring (System Suitability). Because the analytical response in ESI does not correspond to the real concentration of analytes in solution but to the easiness

of their ionization, care must be taken to perform analytical system monitoring to guarantee accuracy and precision. To monitor possible interferences (equipment drift, analyst, etc.), a reference CO sample, whose physical chemical properties were known, was used to monitor the system by running it every

Table 12. Control Chart of the ESI (+) Data in Relative Abundance (%) for the Reference CO Sample

OBS	replicate	analyst	N (%)	N ₂ (%)	NO (%)	NO ₂ (%)	NS (%)
1	1	1	95.0	5.0	0.0	0.0	0.0
2	2		93.5	3.7	2.8	0.0	0.0
3	3		90.5	5.9	1.7	0.8	1.1
4	4		92.3	5.6	1.0	0.0	1.1
5	5		91.5	5.4	2.1	0.0	1.0
6	1	2	90.8	6.1	1.9	1.2	0.0
7	2		90.3	5.3	2.2	1.4	0.8
8	3		88.0	4.9	4.9	1.1	1.1
9	4		91.5	4.2	4.3	0.0	0.0
10	5		88.8	4.6	4.7	0.7	1.2
11	6		93.0	7.0	0.0	0.0	0.0
12	7		88.4	4.7	5.0	0.7	1.2
13	8		87.4	4.8	5.4	1.0	1.4
14	1	3	86.9	5.8	4.2	1.6	1.5
15	2		86.9	6.9	2.9	2.1	1.2
16	3		88.8	6.1	3.3	0.9	0.9
17	4		89.4	6.7	1.4	1.6	0.9
18	1	4	90.5	7.3	1.2	1.0	0.0
19	2		91.3	7.4	1.3	0.0	0.0
20	3		88.7	5.6	1.5	1.4	2.8
21	4		90.4	7.7	1.0	0.9	0.0
22	5		88.7	7.8	1.7	1.0	0.8
23	6		91.3	6.9	1.8	0.0	0.0
24	7		88.5	5.1	5.2	0.0	1.2
25	1	5	88.9	7.0	1.7	1.5	0.9
26	2		94.3	2.5	3.2	0.0	0.0
27	3		91.1	6.5	2.4	0.0	0.0

working day prior to other samples. Table 12 shows the results obtained from the reference CO sample analyzed by ESI (+) FT-ICR MS once a day over a period of five non-sequential weeks by different analysts over 3 months.

Figure 5 shows the control chart results for the N class, in which one can observe no pattern of bias, and the average is situated in the range 87.5–91.43%, despite the spread out results for analyst 5. For the NO class, the results by analyst 4 show a point that exceeds 3σ , but when considering all analysts, this deviation is not a matter of concern. The chart for the individual values obtained via classes detected via ESI (+), as well as the values obtained for the moving range, which are the differences between two consecutive individual values, show good agreement among different analysts.

CONCLUSION

The precision of petroleomic ESI (\pm) FT-ICR MS has been evaluated. Small variabilities were observed, leading in general to excellent coefficients of variance, in the range 5–18% for ESI (+) and in the range 2–7% for ESI (–). Precision, as measured by both repeatability and reproducibility, was found to be affected mainly by differences in the chemical composition of the CO samples, which alters the ionization efficiencies. Interlaboratory comparison has shown good agreement for both the ESI (+) and ESI (–) FT-ICR MS data. Furthermore, the results also suggest that, when applying ESI-FT-ICR MS for petroleomics studies, comparisons should be performed using classes with relative abundances no lower than 10% to achieve proper precision. When ESI (+) is compared to ESI (–), the negative mode was more powerful at distinguishing among samples due to greater variations in relative abundances of the classes detected in this mode.

Precision will be under control if the quality indicators for the spectra profile shape and standard procedures of sample preparation and instrument operation are defined and properly followed. The use of control charts obtained for a most representative reference sample is also a proper tool to guarantee reliability, good performance, and precision of the analytical protocol in FT-ICR petroleomics.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Brazilian Oil Company Petrobras and the Brazilian National Agency of Petroleum, Natural Gas and Biofuels, (ANP) for financial support.

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