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Journal of Chromatography A, 1033 (2004) 83–90
JOURNAL OF CHROMATOGRAPHY A
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Measuring naphthenic acids concentrations in aqueous environmental samples by liquid chromatography

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Received 24 October 2003; received in revised form 16 January 2004; accepted 20 January 2004

Abstract

Naphthenic acids are found in wastewaters from petroleum refineries and oil sands extraction plants. Currently, the concentrations of these toxic carboxylic acids are determined by extracting them into methylene chloride and measuring the absorption of the carboxyl group by Fourier-transform infrared (FTIR) spectroscopy. An improved HPLC method, that is simpler and faster than the FTIR method, was used to detect the 2-nitrophenylhydrazides of the naphthenic acids at concentrations as low as 5 mg l^{-1}. Analyses of 58 oil sands water samples showed that the naphthenic acids concentrations determined by FTIR were on average 11% higher than those determined by HPLC.

Keywords: Environmental analysis; Oil sands; Water analysis; Fourier-transform infrared spectroscopy; Derivatization, LC; Naphthenic acids

1. Introduction

Naphthenic acids are present in most petroleum sources and are found in wastewater streams from petroleum refining [1–3]. These petroleum acids are the major water-soluble organic constituents of bitumen in the oil sands deposits of northeastern Alberta, Canada [4]. With an extraction process based on aqueous digestion, these acids are ubiquitous and account for the majority of the dissolved organic matter in the process-affected waters produced at the oil sands extraction plants in northeastern Alberta [5–7]. Naphthenic acids are complex mixtures of alkyl-substituted acyclic and cycloaliphatic carboxylic acids, with the general chemical formula $C_nH_{2n+Z}O_2$, where n indicates the carbon number and Z is zero or a negative, even integer. When $Z = 0$, the compounds are acyclic, when $Z = -2$, the compounds contain one ring, when $Z = -4$, the compounds contain two rings, and so on. These acids have been shown to be toxic to fishes [8], animals [9,10], and plants [11].

Currently, in most surface oil sands operations, the extraction of bitumen from oil sands uses a modified Clark hot water, caustic-extraction process, in which the oil sands ore is digested with warm (40–80 °C) water and sodium hydroxide (50–200 g/t of oil sands) as a process aid [7]. Under the resulting alkaline conditions (pH 8.5–10.5), the naphthenic acids in the bitumen are solubilized and released into the aqueous phase as sodium naphthenates. At present, the oil sands plants do not release any of the resulting extraction-affected waters from their leases, so that fluid tailings are contained on site, primarily in large settling ponds. The resulting process-affected waters have been shown to have naphthenic acids concentrations in the range of 40–120 mg l^{-1} [7,12–14].

A Fourier-transform infrared (FTIR) spectroscopy method is used most commonly to measure the concentrations of naphthenic acids in oil sands-affected waters. In this method, the acids are extracted from an acidified water sample into methylene chloride, and the absorbance of the carboxylic acids at wave numbers of 1743 and 1706 cm^{-1} are measured using FTIR spectroscopy [15]. A commercially-available naphthenic acid preparation is used to prepare the calibration curve for these quantitative analyses. This method was developed at Syncrude Research, and it has been used extensively to determine naphthenic acids concentrations in various studies [6,7,11,13,14,16,17].

Miwa [18,19] and Miwa et al. [20–22] developed and thoroughly documented high performance liquid chromatography (HPLC) methods for detecting and separating

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doi:10.1016/j.chroma.2004.01.030

the 2-nitrophenylhydrazide derivatives of fatty acids and other carboxylic acids in aqueous solution. Clemente et al. [23] adapted this method to monitor the biodegradation of naphthenic acids by laboratory bacterial cultures. In the FTIR method, quantification of the acids in the naphthenic acid grouping is based on the response of the carboxylic groups. Using a similar rationale, Clemente et al. [23] showed that by derivatizing the carboxyl groups with 2-nitrophenylhydrazine, then separating the derivatized naphthenic acids from the excess reagents by HPLC with detection at 400 nm, a successful quantitation method was possible. Because of the complexity of mixtures of naphthenic acids, the derivatized compounds eluted from the column as a hump of unresolved compounds. The areas under the humps were integrated, and by comparing these to the areas under the humps of known concentrations of derivatized commercial preparations, the biodegradation of naphthenic acids from the microbial cultures was followed more easily than if the FTIR method had been used. However, the reagent blanks, that contained no naphthenic acids, gave large areas under the hump, and the integrations of these were not reproducible. Thus, the HPLC method could not accurately determine naphthenic acids concentrations below about 15 mg l^{-1}. Other researchers have derivatized naphthenic acids to their esters, and analyzed these by gas chromatography, using the areas under the humps to estimated the amounts of naphthenic acids in their samples [24,25].

Because of the acute toxicity of naphthenic acids to many aquatic organisms at concentrations found in process-affected waters, the oil sands companies are required to monitor and report concentrations of naphthenic acids in various waters on and near their leases. Currently, the FTIR method provides this information. In the spring of 2003, Syncrude Canada Ltd. undertook a survey to measure the naphthenic acids in water samples from a variety of locations at their Mildred Lake site (leases 17 and 22). This provided a good opportunity to undertake a direct comparison of the results obtained from the FTIR method with those obtained by the HPLC method.

The initial objective of this work was to improve the minimum detection limit of the previously described HPLC method [23] for the analysis of aqueous solutions of naphthenic acids. This modified procedure was then used to compare the results from the HPLC method with the results from the industry-adopted standard FTIR method for 58 water samples from the oil sands operation at Syncrude Canada Ltd.

2. Experimental

2.1. Naphthenic acid and carboxylic acid standards

Kodak naphthenic acids (lot 115755A) and Kodak naphthenic acids sodium salts (lot B14C) were purchased from The Eastman Kodak Company (Rochester, NY). Refined Merichem naphthenic acids were a gift from Merichem Chemicals and Refinery Services. Naphthenic acids were extracted from 160 l of water collected from the Mildred Lake Settling Basin (MLSB), a large tailings settling pond at the Syncrude oil sands extraction site. Details of this extraction method are given in Holowenko et al. [13,14]. The resulting extract provided naphthenic acids dissolved in an alkaline solution, with a concentration of 3100 mg l^{-1}, as determined by FTIR spectroscopy [17].

Individual solutions of seven reagent quality carboxylic acids, that were considered as surrogate or model naphthenic acids ($C_nH_{2n+Z}O_2$), were prepared by dissolving each acid in 95% ethanol to a final concentration of approximately 200 mg l^{-1}. These acids included cyclohexanebutyric acid, *trans*-1,4-pentylcyclohexanecarboxylic acid, lauric acid and palmitic acid (Aldrich, Milwaukee, WI), stearic acid (BDH Chemicals Ltd., Poole, UK), and 5β-cholanic acid (Sigma, St. Louis, MO). These were derivatized and analyzed by HPLC to determine their retention times.

Commercial and environmental naphthenic acids standard curves were prepared by dissolving naphthenic acids in 0.1 M NaOH to make a 2000 mg l^{-1} solution. This stock solution was further diluted with appropriate volumes of 0.1 M NaOH and MilliQ water to yield naphthenic acids standards with a final NaOH concentration of 0.01 M.

2.2. HPLC method: derivatization step

The reaction mixture consisted of 200 μl of alkaline naphthenic acids standard or sample, 80 μl of 2-nitrophenylhydrazine (2-NPH, ICN Biomedical Inc., Aurora, OH) solution, 80 μl of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (1-EDC-HCl, Sigma) solution, all contained in a sealed 1.5 ml, glass, screw-cap vial. The 2-NPH solution was prepared by dissolving 60 mg 2-NPH in 15 ml of 95% ethanol and 5 ml of 0.4 M HCl. The 1-EDC-HCl solution was prepared by dissolving 480 mg of 1-EDC-HCl in 10 ml of 95% ethanol and 10 ml of 3% pyridine in 95% ethanol. The reaction mixture was incubated for 20 min in a 60 °C water bath. The vials were removed from heat and 40 μl of 140 mM KOH (prepared in 80% (v/v) HPLC grade methanol in MilliQ water) was added to the mixture. The vials were then incubated for another 15 min in the 60 °C water bath and then cooled in a cold water bath.

2.3. HPLC method: HPLC analysis

The HPLC was an Agilent (Wilmington, DE) 1100 Series HPLC with an autosampler, thermostated column compartment, UV-visible diode array detector and a degasser. The Agilent Chemstation used software for an LC-3D system. The HPLC had a guard column and an analytical column. The guard column was packed with 2 μm RP-18 solid phase, and the analytical column was an Agilent LiChrospher 100 RP-18 column (5 μm particle size, 125 mm × 4 mm). The

analytical column was kept at 40 °C and the sample injection volume was 60 μl. The mobile phase was a programmed mix of HPLC grade methanol (Fisher Chemicals, Fairlawn, NJ) and MilliQ water. The mobile phase was run on a gradient from 70:30 methanol:water at the time zero, to 100% methanol at 4 min, with a flow rate of 1.5 ml min^{-1}. The total run time was 7 min, followed by 4 min of post-time to prepare the HPLC for the next injection. The detector was set at 400 nm (bandwidth of 10 nm), with a reference wavelength at 510 nm (60 nm bandwidth). Integration was done using baseline hold.

2.4. Field samples from Syncrude's leases 17 and 22

In June 2003, 58 water samples were collected from the Syncrude's Mildred Lake site in northeastern Alberta, Canada. Extraction tailings, including water, sand, clays and unrecovered bitumen, have been deposited in the MLSB since 1978, with the saturated sand being used to construct the dykes and beaches that form the containment system. A further sand addition, the East Toe Berm, was added externally in the northeastern area of the MLSB, close to a creek valley, Beaver Creek. Waters contained within the saturated sand deposits have been slowly seeping from both structures since their deposition. A seepage control system with ditches and collection ponds has been established to prevent direct discharge of these sand seepage waters. By using this system, most of the released process-affected waters is returned to the MLSB. The intent of the operation is that the waters associated with tailings deposits (tailing ponds, beaches, dykes, berms, and seepage collection ditches) are collected through engineered systems to ensure that the naphthenic acids-containing, process-affected waters are contained on the lease, rather than discharged. The major focus of the current sampling program was to determine naphthenic acids concentrations in waters in the neighbouring Beaver Creek Valley and seeps feeding the system. Beaver Creek was sampled from the perimeter of the MLSB, to approximately 1 km beyond the boundary of the Syncrude lease. Seeps along the valley wall were sampled between the MLSB and a lower seepage dam. A number of other samples were taken including the waters from the seepage control ditches, the main settling basins (MLSB, West-In Pit), seepage control pond, and the East Toe berm seepage controls. Groundwaters from the same area were also collected. In total, there were 22 creek samples, 14 seep samples, 2 tailing pond samples, 9 groundwater samples, and 11 seepage control pond and ditch samples.

2.5. Field sample preparation for HPLC analysis

The pH of a 5-ml portion of an aqueous sample was adjusted to approximately 12 by the addition of a few drops of 2 M NaOH. The sample was drawn into a 5-ml Luer Lok syringe and filtered through a 2.5-cm diameter Millipore (Billerica, MA) 0.22 μm GV filter housed in a Millipore Swinnex-25 filter holder. The pH of the filtrate was adjusted between 8 and 10 with 3 M HCl, and three 200-μl portions were dispensed into 1.5 ml, glass screw-cap vials for derivatization. The reported results are means of the triplicate analyses.

2.6. Field sample preparation for FTIR analysis

The aqueous samples (100–200 ml) were adjusted to a pH of 2.0–2.5 using 9 M H_2SO_4. The acidified sample was extracted twice with 20-ml portions of methylene chloride (Optima grade, Fisher Chemicals). The extracts were combined in a 50-ml screw top test tube and then taken to dryness overnight under a flow of compressed air. Prior to FTIR analysis, an accurately weighed amount of methylene chloride (7–13 g) was added to the dried sample. The resulting solution was transferred to a 5 ml cuvette to yield a concentration of 25–400 mg of naphthenic acids per kg of methylene chloride (about 125–2000 μg naphthenic acids) that was required for the FTIR analysis.

2.7. FTIR step

The FTIR spectra were obtained using a Nicolet Magna-IR™ 550 Spectrometer with a variable length KBr cell chamber set to 3 mm. The OMNIC E.S.P. 5.1 software was used in conjunction with the FTIR instrument. The software parameters were set to 128 scans and view limits of 1850–1650 cm^{-1}.

A background scan of methylene chloride in the KBr cell was collected each day prior to analysis. The instrument sample chamber was purged under dry air for 15 min to remove moisture prior to the background scan. The software automatically applied this background scan to subsequent sample scans. Samples dissolved in methylene chloride were placed in the KBr cell in the sample chamber and the chamber was purged under dry air for 5 min. After the scans were completed, the two peaks of interest were summed and then compared to the calibration curve prepared with Kodak naphthenic acids using the combined peak heights. The two selected peaks represent the monomer (1743 cm^{-1}) and the dimer (1706 cm^{-1}) forms of carboxylic acids.

3. Results and discussion

3.1. Modifications to and characterization of the HPLC method

In Table 1, the comparison of features of the original HPLC method of Clemente et al. [23] and the modified HPLC method is presented. Two modifications were made to the derivatization procedure. The compositions of two of the reagent solutions was altered, and the volumes of the sample and reagents in the reaction mixture were changed. The derivatization reagents were modified, specifically the

Table 1
Comparison of methods for naphthenic acids analysis using the original HPLC method of Clemente et al. [23] with the modified version in present study

Step	Method of Clemente et al. [23]	Modified method
Reagents	0.02 M 2-NPH in 0.05 M HCl EDC-HCl 69 mM KOH in 80:20 MeOH:H_2O	0.02 M 2-NPH in 0.1 M HCl No change 140 mM KOH in 80:20 MeOH:H_2O
Reaction mixture	50 μl aqueous sample 100 μl 0.02 M 2-NPH in 0.05 M HCl 100 μl EDC-HCl 50 μl 69 mM KOH	200 μl aqueous sample 80 μl 0.02 M 2-NPH in 0.1 M HCl 80 μl EDC-HCl 40 μl 140 mM KOH
Reaction condition	Incubate reaction mixture for 20 min at 60 °C. Remove from heat, add KOH solution and heat for 15 min at 60 °C. Cool in water bath	No change
HPLC method		
Injection volume	60 μl	No change
Column temperature	40 °C	No change
Solvents	90 μl 0.185 M phosphoric acid in 1 l methanol (solvent A) 28 μM phosphoric acid in MilliQ water (solvent B)	Methanol MilliQ water
Solvent program	70% solvent A, 30% solvent B from 0 to 1.8 min and 100% solvent B after 2 min	Gradient from 70:30 methanol:water to 100% methanol from 0 to 4 min and 100% methanol after 4 min
Flow rate	1.5 ml min^{-1}	No change
Total run time	7 min	No change
Integration time	2.9–6.0 min	No change
Detection limit	∼15 mg l^{-1}	∼5 mg l^{-1}

2-NPH solution and the KOH solution. The 2-NPH was dissolved in ethanol and 0.4 M HCl, resulting in a final acid concentration of 0.1 M in the solution. The modified solution contains twice the concentration of acid as the original solution. Subsequently, the concentration of the KOH solution was also doubled, to 140 mM, to compensate for the increased acid in the reagent mixture. By increasing the acid and base concentrations, greater control of the pH of the reaction mixture was achieved. The pH of the reaction mixture, after the addition of KOH, is very important because it influences the absorption wavelengths of the derivatized naphthenic acids. Below pH 8, the derivatized solution is orange and absorbs maximally at 400 nm, but at pH greater than 10 the solution turns purple, with reduced absorption at 400 nm [19].

In the modified method, five volumes of naphthenic acids sample were reacted with two volumes of 2-NPH solution, two volumes of 1-EDC-HCl solution and one volume of KOH solution. This reaction resulted in a two-fold dilution of the sample, whereas in the original HPLC method, the sample was diluted six-fold (one volume of sample, two volumes of 2-NPH solution, two volumes of 1-EDC-HCl solution and one volume of KOH solution). The change in sample and reagent volumes resulted in naphthenic acids standard curves with slopes that were about twice as large as those obtained by the original method. This increased slope improved the sensitivity of the modified HPLC method.

The mobile phase and the solvent gradient were modified from original method (Table 1). Experience showed that there was no difference between calibration curves prepared with or without phosphoric acid in the mobile phase, so this acid was omitted from the methanol and MilliQ water. In the original method, solvent A was pumped for the first 1.8 min followed by a rapid change to solvent B by 2.0 min. This quick change in solvents gave a disturbance in the baseline when no sample was injected, contributing to the area of the hump obtained from the derivatized naphthenic acids. In the modified method, a gradient was used over 4 min of elution. The mobile phase started as 70:30 methanol:MilliQ water, changing to 100% methanol over 4 min. This produced much less baseline disturbance.

Fig. 1 shows the output of the HPLC detector at 400 nm. The minor baseline disturbance obtained with the mobile phase program without sample injection is shown in Fig. 1C. This change in baseline is a result of the mixing of solvents in the mobile phase. Clemente et al. [23] used an abrupt change in solvents, which caused a disturbance between 3 and 4 min, that appeared as two poorly resolved peaks with height of about 7 mAU. In contrast, the solvent gradient used in the modified method produced a gentle slope between 2.5 and 5.2 min, with a maximum absorbance of about 3 mAU (Fig. 1C) and a typical area count of 170 between 2.9 and 6.0 min.

Fig. 1B was obtained from a 60 μl injection of a reagent blank that contained no naphthenic acids, but contained all of the reagents. There were five distinct peaks on the chromatogram before 2.9 min (numbered 1–5). Injections of individual derivatizing reagents showed all of these peaks were from the 2-NPH solution. The typical area count from 2.9 to 6 min was 400.

Fig. 1A shows the chromatogram obtained from a 60 μl injection of a solution containing derivatized Kodak

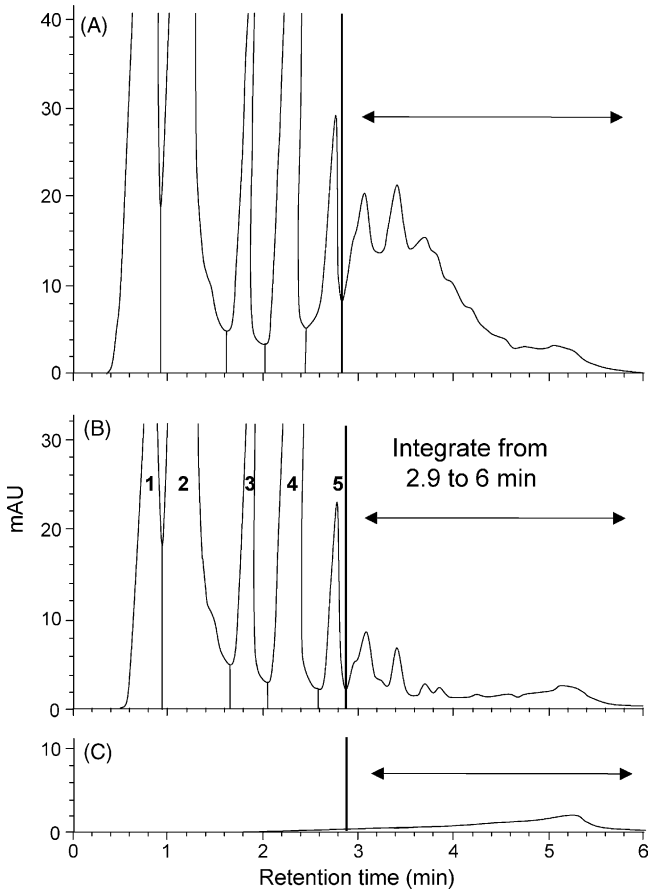

Fig. 1. HPLC chromatograms obtained from injections of (A) 60 μl of derivatized Kodak naphthenic acids (100 mg l^{-1}); (B) 60 μl of reagent blank with no naphthenic acids; (C) 0 μl of sample. Integration was done using a baseline hold.

naphthenic acids (100 mg l^{-1}). The large unresolved hump after peak 5 contains the derivatized naphthenic acids. A valley appeared after the elution of peak 5 (at 2.9 min) and the total area of the hump between 2.9 and 6.0 min was integrated as the naphthenic acids (area count 1440). Fig. 2 shows a typical calibration curve obtained by derivatizing solutions of Kodak naphthenic acids with concentration of 5–100 mg l^{-1}. The *y*-intercept was 348 area counts. The

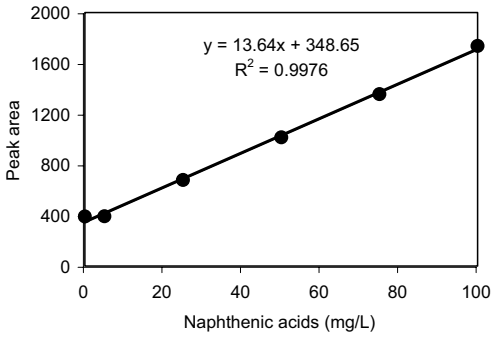

Fig. 2. Calibration curve obtained with derivatized Kodak naphthenic acids.

y-intercept was always above zero because there are materials in the reagent blank that elute between 2.9 and 6.0 min (Fig. 1B). The baseline disturbance associated with the solvent gradient (Fig. 1C) contributes nearly one-half of the area count in the reagent blank. The modified method typically gave *y*-intercept values of 200–400 area counts, whereas the *y*-intercepts reported by Clemente et al. [23] were between 500 and 900 area counts. The modified HPLC method had increased sensitivity and decreased hump area in the blank analysis (apparent as the reduced area count of the calibration curves). This lowered the minimum detection limit to between 5 and 10 mg naphthenic acids per litre. No attempt was made in the present study to concentrate samples using other methods, such as using solvent extraction and concentration or using solid-phase extraction [25].

To assess the approximate molecular weight range of naphthenic acids that would elute between 2.9 and 6.0 min, seven carboxylic acids, considered as model naphthenic acids, fitting the formula $C_nH_{2n+Z}O_2$, were individually derivatized and analyzed by HPLC. The seven compounds were cyclohexanebutyric acid, *trans*-1,4-pentylcyclohexane carboxylic acid, lauric acid, palmitic acid, stearic acid and 5β-cholanic acid (with underivatized molecular weights of 170.2, 198.3, 200.3, 256.4, 384.5 and 360.6, respectively). These represented acids with $n = 10$ (cyclohexanebutyric acid) to $n = 24$ (5β-cholanic acid). The derivatized cyclohexanebutyric acid had a retention time of 2.7 min and it co-eluted with peak 5. The remaining six model acids eluted within the interval of 2.9–6 min used to integrate the area under the naphthenic acids hump.

Fig. 3 shows the chromatograms of three additional commercially-available naphthenic acids preparations (about 75 mg l^{-1}). These chromatograms, and Fig. 1A, illustrate that the humps from various naphthenic acids preparation essentially return to the baseline by 6 min. This indicates that there are likely few, if any, higher molecular weight acids eluting after 6 min that would escape integration. In contrast, the work with the model naphthenic acids indicated that derivatized acids with $n \leq 10$ would elute prior to the start of integration, co-eluting with peak 3, 4 or 5. The molecular weight distributions of several naphthenic acids preparations have been characterized by GC-MS analyses [6,26]. These show that the relative abundance of ions, corresponding to $n \leq 10$, is generally small. For example, 6% of the ions detected in the Kodak naphthenic acids (used in Fig. 1A) corresponded to acids with $n \leq 10$ (unpublished results). GC-MS analyses of naphthenic acids in two oil sands tailings ponds (designated MLSBF and Pit 5) showed that about 7–8% of the ions detected corresponded to acids with $n \leq 10$ [26]. Similarly, 7% of the ions corresponding to acids with $n \leq 10$ were detected in naphthenic acids extracted from an oil sands ore [6]. Thus, only a small portion of the acids in these naphthenic acids mixtures would not be quantified as part of the hump produced by this HPLC method. A notable exception is the Merichem preparation in which 18% of the ions detected

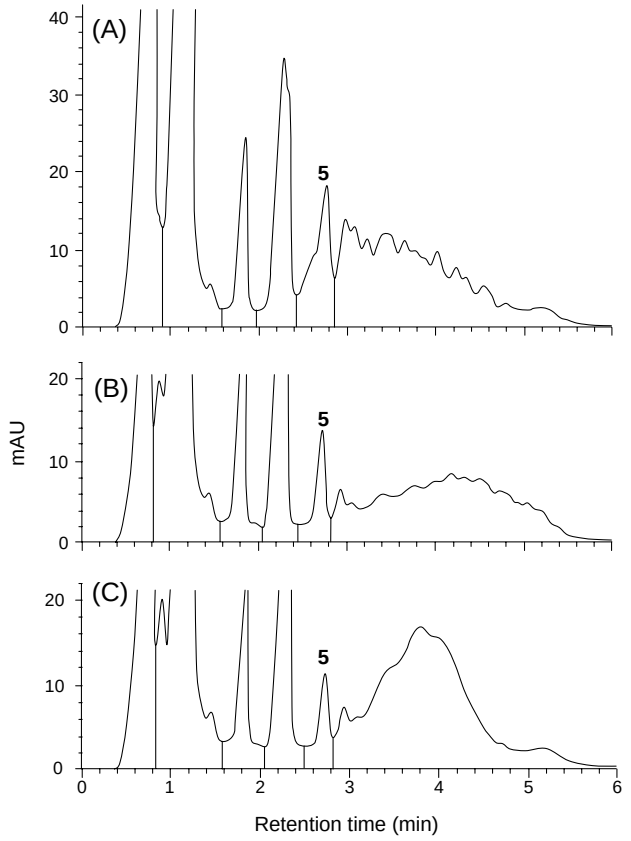

Fig. 3. HPLC chromatograms of derivatized acids from two commercial sources and from the oil sands tailings pond: (A) refined Merichem naphthenic acids; (B) Kodak naphthenic acids salts; (C) naphthenic acids extracted from MLSB. Each solution contained approximately 75 mg of each preparation per litre.

in the GC-MS characterization corresponded to acids with $n \leq 10$ (unpublished results). The shoulder on the leading edge of peak 5 in Fig. 3A gives this peak a unique shape, which is likely due to the abundance of acids with $n \leq 10$.

Each HPLC chromatogram in Fig. 3 shows a different hump shape eluting after peak 5, and these are different than the hump shape given by the Kodak acids (Fig. 1A). Using a GC-MS method, Clemente et al. [26] characterized several naphthenic acids preparations based on the distributions of ions that corresponded to naphthenic acids with different n values, Z numbers, and molecular weights. They then applied a statistical analysis to determine which preparations were different. They reported that Merichem acids differed from the Kodak salts, and that the Kodak salts differed from the Kodak acids. These different molecular weight distributions likely contribute to the different shapes of the humps shown in Fig. 1A and 3.

3.2. Comparisons between the FTIR and HPLC methods

A naphthenic acids concentrate, that had been extracted from Syncrude process waters, and was used in a previous study [14], was diluted in MilliQ water and analyzed by both the FTIR and HPLC methods (Table 2). There was a good agreement between the concentrations measured by the two methods, with the HPLC method tending to give slightly higher values. The slope of the line obtained by plotting the FTIR results as the ordinate, and the HPLC results as the abscissa was 0.93, with a correlation coefficient of 0.976.

Table 2
Comparison of FTIR and HPLC analyses of naphthenic acids extracted from MLSB and diluted in MilliQ water

Estimated concentration ($mg\,l^{-1}$)[a]	FITR results ($mg\,l^{-1}$)[b]	HPLC results ($mg\,l^{-1}$)[c]
0	0.5	4 ± 2.5
10	8	13 ± 1.8
30	24	22 ± 2.1
60	51	64 ± 2.6
80	75	78 ± 0.5

[a] The extract contained approximately 3100 mg naphthenic acids $mg\,l^{-1}$ by FTIR analysis [14].
[b] Results from a single analysis.
[c] Mean and standard deviation of triplicate analyses.

When working with the environmental samples, we wanted to use a minimum amount of sample preparation prior to HPLC analysis. Before filtering the environmental samples, their pH was adjusted to approximately 12 to ensure that the naphthenic acids were in their soluble naphthenate forms. Miwa [19] stressed that the pH of the reaction mixture was important. If the pH was too high, the yield of carboxylic acid hydrazides was decreased. To avoid this problem, the pH of the environmental samples was adjusted to between 8 and 10 after filtration, prior to the derivatization reaction, and the concentration of HCl in the 2-NPH solution was increased (Table 1).

The water from MLSB contains dissolved organic residuals from the oil sands extraction process, so it was chosen to determine if there were any interfering materials that would elute with retention times between 2.9 and 6 min. A 60-μl filtered sample of this water, which had not been derivatized, was injected into the HPLC. The effluent was monitored at 280 and 400 nm (the latter being the wavelength used to monitor the derivatized naphthenic acids). At 280 nm, it was apparent that some materials from the MLSB sampled eluted from the HPLC column, but at 400 nm, there was little material detected, and the detector output was similar to that shown in Fig. 1C. Nearly identical results were obtained when a sample of underivatized Kodak acids ($100\,mg\,l^{-1}$) was analyzed in the same manner with the detector set at 280 or 400 nm. Thus, it was concluded that other than filtration (0.22 μm pore size) to remove any suspended materials to protect the HPLC column, no additional cleanup was need for the environmental samples prior to derivatization.

The Kodak acids were used to prepare the calibration curves for both the FTIR and HPLC methods. Fig. 4 compares the concentrations of naphthenic acids obtained by FTIR and HPLC analyses of 58 water samples collected from the Syncrude lease. If results for each sample were identical by both methods, all of the data points would fall on the

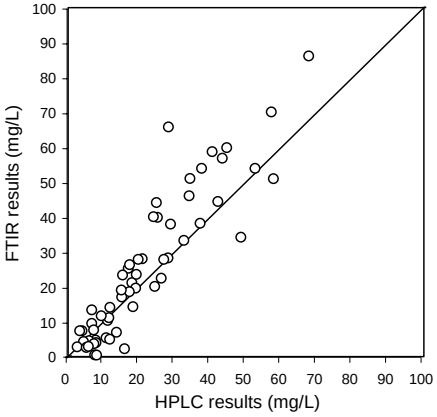

Fig. 4. Naphthenic acids concentrations obtained from the analyses of 58 water samples by FTIR and HPLC. If the two methods gave identical results, all the points would fall on the diagonal equivalence line that is shown.

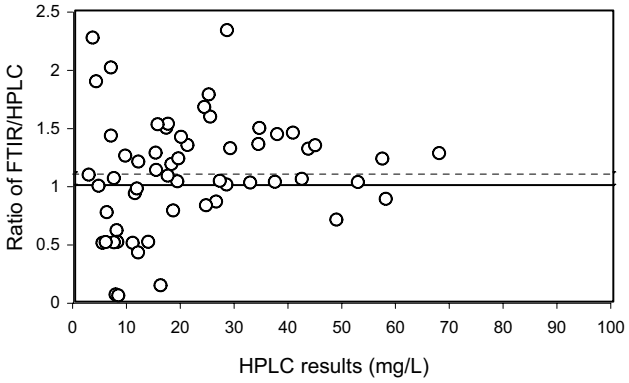

Fig. 5. Ratio of naphthenic acids concentration measured by FTIR to that measured by HPLC compared to the concentration measured by HPLC.

equivalence line. In general, there was good agreement between the two methods. Unlike the results in Table 2, the FTIR method gave slightly higher concentrations in many cases, as shown in Fig. 4.

Due to the analysis time, expense and in some cases because of the large volume of sample required, results of the FTIR analyses shown in Fig. 4 are from a single analysis of each water sample. In contrast, triplicate analyses of each water sample were done with the HPLC method, and the means are plotted in Fig. 4. The reproducibility of the triplicate analyses was excellent for 43 samples that contained >10 mg naphthenic acids per litre. The relative standard deviations for these samples ranged from 0 to 14%, with a mean of 0.9%. In contrast, the relative standard deviations for the 15 samples that contained <10 mg naphthenic acids per litre were higher. These ranged from 0.3 to 73%, with a mean of 28%.

During the development and application of the FTIR method, the Kodak naphthenic acid preparation was used to assesses the accuracy and reproducibility of the method. Replicate analyses of a 100 mg l^{-1} standard solution, the FTIR method gave consistent and reliable results (99 ± 6 mg l^{-1}). The major issue regarding the FTIR method is potential for contamination from materials such as phthalic acids during extraction or in the solvents, and the presence of non-naphthenic acid carboxylic acids in the extracts. The FTIR method is very susceptible to these interferences.

Fig. 5 summarizes the ratios of the concentration measured by FTIR to the concentration measured by HPLC for each sample, plotted against the concentration measured by HPLC. The greatest differences between the two methods were observed at concentrations below 30 mg l^{-1}. If the two methods yielded identical results, all of the calculated ratios would have a value of 1.0. Considering the data from the 58 samples, the average ratio was 1.11 (shown as a broken line in Fig. 5) with a standard deviation of 0.48.

Because of the complexity of the composition of naphthenic acids and the nature of these two analytical methods, it is not possible to determine which method provides the more accurate results. Both methods rely on the carboxylic groups for quantification and assume that the Kodak naphthenic acids preparation serves as a suitable calibration standard for the naphthenic acids in environmental samples. The FTIR method assumes that the only carboxylated compounds extracted from an aqueous sample into the methylene chloride are naphthenic acids and the naphthenic acids are quantitatively extracted into the organic solvent. The HPLC method assumes that the only carboxylated compounds that react with the 2-NPH are naphthenic acids and that all of the acids react completely with the derivatizing agent. Deviations from any of these assumptions will produce biased results.

The HPLC method has several advantages over the FTIR method. For example, the sample volume required for the HPLC method is much smaller than the volume required for the FTIR method. A 5-ml sample is adequate for the HPLC method that requires only 200 μl in the derivatizing reaction. In contrast, volumes of up to 200 ml were required for the lower naphthenic acid content water samples from Beaver Creek. In situations where collecting and transporting larger sample volumes are a challenge, the volume required at the analytical stage may be important.

The time required to prepare and analyze samples by HPLC is much less than that required for the FTIR method. The personnel time for the preparation of 30 samples (in triplicate) for HPLC analysis is about 5 h. The analyses of these sample can be completed overnight using an unattended, automated HPLC system. The methylene chloride extractions are more time consuming, and about 30 samples (without replication) can be extracted in a 5-h period. An overnight evaporation is required to remove the methylene chloride from the extract prior to final preparation for the FTIR analysis. Each FTIR analysis takes about 10 min. Thus, an additional 5 h of personnel time is required for the analyses of 30 samples by FTIR.

The results from the HPLC tended to be lower than those obtained by the FTIR method (Fig. 5), although at this stage it is not possible to know which method is the more accurate. The minimum detection limit for the HPLC method is between 5 and 10 mg l^{-1}, whereas the detection limit for the FTIR is lower because of the extraction and concentration steps. A similar solvent extraction and concentration procedure could be done prior to HPLC analysis to improve the detection limit of the method. Alternatively, the concentration step could be done by solid-phase extraction [25]. Nonetheless, at this stage of the analytical development, the HPLC method offers an attractive alternative to the FTIR method, mainly because of smaller sample volumes, faster sample preparation, and automated HPLC analyses which means that much less personnel time is required. In addition, the use of a chlorinated solvent is avoided with the HPLC method. These advantages lend themselves to degradation and attenuation studies in which time series and various treatment scenarios will result in a large number of samples. The HPLC method is well suited to such studies and will provide better efficiency and ultimately more data on the pathways and fate of the naphthenic acids under environmental and laboratory conditions.

Acknowledgements

Funding for this project was provided by Syncrude Canada Ltd., Suncor Energy Inc., TrueNorth Energy, Albian Sands Energy, Canadian Natural Resources Limited, and the Canadian Water Network. The Natural Sciences and Engineering Research Council of Canada provided funding to purchase the HPLC. The technical support of Allan Yeung at Syncrude Research Department in conducting the FTIR analyses was appreciated. We thank Joyce Clemente for her guidance during this study.

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