Separation of thia-arenes and aza-arenes from polycyclic aromatics in snowpack samples from the Athabasca oil sands region by GC×GC/ToF-MS

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ABSTRACT

Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC/ToF-MS) was used for the analysis of thia-arenes and aza-arenes in standard mixtures containing 45 polycyclic aromatic compounds (PACs), and in the aromatic fraction of snowpack samples collected from the Athabasca oil sands area of Alberta, Canada. The GC columns used included a shape-selective liquid-crystalline stationary phase (LC-50) and a high-polarity ionic-liquid stationary phase (SLB-IL60), both in the first dimension. A fast diffusion and low-polarity nano-stationary phase (NSP-35) and a mid-polarity stationary phase (Rxi-17), were used in the second dimension, respectively. Both configurations showed good repeatability for retention times in the first and second dimensions, peak areas and peak heights. Instrument detection limits ranged from 0.5 to 10 pg µL⁻¹ for both configurations. In general, the LC-50×NSP-35 configuration favoured the separation of isomeric compounds by using more of the 2D chromatographic space available (>80%), particularly for compounds with molecular mass >160 u. Therefore, LC-50×NSP-35 was recommended for the analysis of thia-arenes and alkylated PAHs in environmental samples collected from the oil sands area. Alternatively, the SLB-IL60×Rxi-17 configuration favoured molecular similarity grouping over isomer separation. This was observed for a group of aza-arenes (i.e. carbazoles, benzo[α]carbazoles and alkylated-derivatives), which were resolved from other PACs and from the sample matrix. The risk of false positives and overestimations in the analysis of thia-arenes, aza-arenes and alkylated PACs in one-dimension GC/MS was explored and further reduced by using GC×GC/ToF-MS with LC-50×NSP-35 and SLB-IL60×Rxi-17.

1. Introduction

Polycyclic aromatic compounds (PACs) are a large and diverse class of organic contaminants produced by the incomplete combustion of organic matter through natural or anthropogenic processes [1]. PACs are ubiquitous in the environment and can form complex mixtures of a wide range of congeners with different molecular weights and...
structural types. These mixtures can include PACs with only carbon and hydrogen atoms in their structure, such as unsubstituted polycyclic aromatic hydrocarbons (unPAHs) and alkylated PAHs (aPAHs), and also heteroaromatic compounds in which one or more carbon atoms are replaced by nitrogen (aza-arenes) or sulphur atoms (thia-arenes) [1–3]. Some unPAHs have been classified as toxic, carcinogenic and mutagenic [4–8], and therefore can pose risks to human health and the environment. Similarly, several aPAHs have also shown to be important contributors to the total toxicity of mixtures, in some cases reaching as much as 80% of the total toxic burden [9,10]. Additionally, aPAHs are commonly used in forensic research as indicators of petroleum, coal tar and creosote weathering in the environment [10–13]. Therefore, an accurate identification and estimation of aPAHs is of particular interest. Aza-arenes and thia-arenes are formed in most of the same processes as unPAHs and aPAHs, and can also be found in fossil fuels, automobile exhaust and urban atmosphere [1,14–18]. Aza-arenes are of particular interest to industry because some congeners can lead to poisoning of refining catalysts, promoting corrosion and gum formation in fuels [19]. Similarly, the removal of thia-arenes and organic sulphur compounds is of concern to the coal industry [19]. Although it is well known that some aza-arenes and thia-arenes have shown mutagenic properties [20–22] and many are classified as known or suspected carcinogens [1,23], they have generally been ignored in risk assessment and environmental analysis, and therefore their speciation in the environment remains largely unknown [24–26].

The Athabasca oil sands of Alberta, Canada, are considered the world’s third largest oil reserve [27]. They contain ~1.7 trillion barrels of bitumen, of which ~170 billion are recoverable with existing technology [28]. PACs in the area can be emitted from a large list of potential sources that include natural erosion of geological formations, forest fires, bitumen upgrading, diesel combustion and airborne dust from mining operations. Therefore, complex mixtures of PACs can be found in several environmental compartments, including lake sediments, snow, and air samples collected from the area [29–34]. Although they are not commonly reported, thia-arenes and aza-arenes can be potential important components of these mixtures.

Traditionally, gas chromatography with mass spectrometry (GC/MS) has been used to identify and quantify PACs in complex samples [35]. However, factors related to the complexity of the sample (particularly for samples containing thia-arenes and aza-arenes), such as matrix interferences, the presence of multiple PAC isomers sharing similar mass fragmentation patterns (i.e. common ion effect), the lack of authentic standards to represent all PACs, and the use of single ion fragments for positive identification (i.e. selected ion monitoring ‘SIM’ in GC/MS) can further complicate the analysis and result in potential errors in the identification and quantitation of some PACs. False positives, overestimations and underestimations have been reported to occur [10,26,36,37]. Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC/ToF-MS) is a good alternative to improve the analysis of complex environmental samples containing thia-arenes and aza-arenes. GC×GC/ToF-MS is a multidimensional technique in which the entire sample is analysed utilising two independent GC columns with different stationary phases and selectivities connected through a modulator [38]. The separation produced by each GC column is maintained throughout the run, and therefore the resolving power of the combined separation exceeds those of the individual stages [39]. At the detector stage, the time-of-
flight mass spectrometer produces complete mass spectra for every ion pulse generated in the ionisation source, allowing all ion fragments to be simultaneously monitored and thereby eliminating the need for SIM methods for screening proposes, although a decrease in sensitivity is observed [40]. Additionally, it has been shown that the chromatographic resolution of complex samples in GC×GC/ToF-MS can be further improved by using non-traditional GC stationary phases [41–43], and by matching their selectivities with specific properties of the analytes of interest [39]. GC×GC has been used for the identification of aza-arenes in heavy gas oil samples [16,17], heavy petroleum fractions [44] and unfractionated coal samples [18]; as well as for the analysis of thia-arenes in coal tar samples [45]. In most cases, the resulting 2D-chromatograms have shown ordered elution patterns that allowed chemical compound classifications based on molecular similarities, and identification through visual inspection [16,18]. Although this approach can be useful to characterise the general complexity of the sample, the chromatographic separation of individual isomers and their separation from the sample matrix are still two challenging tasks, and several co-elutions are reported [17,26,45].

This study focused on the use of GC×GC/ToF-MS for the analysis of thia-arenes and aza-arenes in standard solutions and in the aromatic fraction of snowpack samples collected in the Athabasca oil sands region of Alberta. Non-traditional stationary phases, such as a shape-selective liquid-crystalline (LC-50) and high-polarity ionic-liquid (SLB-IL60), were used. Unlike other studies [46–50], LC-50 and SLB-IL60 were both used in the first dimension, due to their relatively low operational temperature limits (270°C and 300°C, respectively), and to take advantage of their unique selectivity over longer run times. A low-polarity nano-stationary phase (NSP-35) and a mid-polarity (Rxi-17) were used in the second dimension, respectively. The LC-50×NSP-35 configuration has shown to be effective in the separation of a large number of PACs [41,43], and was used in this study to improve the resolution of thia-arenes. The SLB-IL60×Rxi-17 configuration was used to increase the selectivity towards relatively more polar and less abundant aza-arenes, in order to resolve them from the sample matrix and other sample components.

2. Experimental

2.1. PAC standard solution

Method optimisation was carried out using standard solutions containing 45 PACs in toluene at concentrations ranging from 0.5 to 500 pg µL \(^{-1}\). The standard mixtures included 20 unPAHs, 15 aPAHs, 6 thia-arenes and 4 aza-arenes (Table 1). Standards were purchased from Chem Service (West Chester, PA, USA), Sigma-Aldrich (St. Louis, MO, USA), AccuStandard (New Haven, CT, USA) and Chiron (Trondheim, Norway).

2.2. Snowpack sample collection and processing

The snowpack samples analysed were collected as part of a larger study focusing on contaminants in the snow of the Athabasca oil sands area of Alberta [51]. The samples used in this study were collected during late February and early March 2012 in the Regional Municipality of Wood Buffalo in Northern Alberta, at varying distances from the major industrial developments. Further sampling details can be found in the
supporting information. All samples were kept frozen until further processing at the Environment and Climate Change Canada (ECCC), Canada Centre for Inland Waters (CCIW) in Burlington, Ontario. Melted snow (4–5 L per sample) was filtered by pumping through a 0.7 µm pore size GF/F filter, with 142 mm in diameter and pre-combusted at 400°C. The filter was connected to a 30 cm Teflon column (2 cm i.d.) packed with 50 g of pre-cleaned XAD-2 resin to collect both the dissolved and particulate fractions. Filters were then packed in a 33 mL extraction cell between two layers of 2.5 g of Hydromatrix (diatomaceous earth sorbent), and extracted using a 1:1 hexane-acetone mixture followed by 100% dichloromethane (DCM) in an accelerated solvent extraction system (ASE). The

| PACs     | Abbreviation | Group       | Quantitative ion | Confirmation ions (%) |
|----------|--------------|-------------|------------------|-----------------------|
| 1        | Naphthalene  | NAP         | 128              | 129 (11) 127 (11)     |
| 2        | Acenaphthene | ACY         | 152              | 153 (15) 151 (14)     |
| 3        | Acenphthene  | ACE         | 153              | 154 (83) 152 (51)     |
| 4        | Fluorene     | FL          | 166              | 165 (84) 167 (14)     |
| 5        | Anthracene   | AN          | 178              | 179 (16) 176 (14)     |
| 6        | Phenanthrene | PH          | 178              | 176 (20) 179 (15)     |
| 7        | Pyrene       | PY          | 202              | 203 (17) 200 (15)     |
| 8        | Fluoranthene | FLT         | 202              | 203 (17) 200 (15)     |
| 9        | Benzo(b)fluorine | BbFL        | 216              | 215 (72) 217 (17)     |
| 10       | Triphenylene | TRI         | 228              | 226 (23) 229 (20)     |
| 11       | Chrysene     | CHR         | 228              | 226 (27) 229 (20)     |
| 12       | Benzo(a)anthracene | BaAN      | 228              | 226 (28) 229 (20)     |
| 13       | Perylene     | PER         | 252              | 253 (22) 266 (22)     |
| 14       | Benzo(b)fluoranthene | BbFLT   | 252              | 253 (22) 250 (18)     |
| 15       | Benzo(a)fluoranthene | BkFLT    | 252              | 253 (21) 250 (21)     |
| 16       | Benzo(a)pyrene | BaPY       | 252              | 253 (27) 250 (17)     |
| 17       | Benzo(c)pyrene | BePY       | 252              | 250 (22) 253 (22)     |
| 18       | Benzo(g,h,i)perylene | BghiP   | 276              | 277 (24) 229 (24)     |
| 19       | Dibenzo(a,h)anthracene | DahA    | 278              | 279 (24) 276 (17)     |
| 20       | Picene       | PIC         | 278              | 279 (24) 276 (23)     |
| 21       | 2,3-dimethylnaphthalene | 2,3-metNAP | 141              | 156 (98) 155 (29)     |
| 22       | 2-methylnaphthalene | 2-metNAP  | 142              | 141 (83) 115 (36)     |
| 23       | 1-methylnaphthalene | 1-metNAP  | 142              | 141 (86) 115 (38)     |
| 24       | 2,6-dimethylnaphthalene | 2,6-metNAP | 156              | 141 (75) 155 (42)     |
| 25       | 2,7-dimethylnaphthalene | 2,7-metNAP | 156              | 141 (47) 155 (33)     |
| 26       | 1,5-dimethylnaphthalene | 1,5-metNAP | 156              | 141 (58) 155 (28)     |
| 27       | 1-methylfluorene | 1-metFL    | 252              | 253 (22) 250 (18)     |
| 28       | 1-methylphenanthrene | 1-metPH   | 252              | 253 (21) 250 (21)     |
| 29       | 9-methylanthracene | 9-metAN   | 252              | 253 (27) 250 (17)     |
| 30       | 2-methylanthracene | 2-metAN   | 252              | 253 (27) 250 (17)     |
| 31       | 3,6-dimethylnaphthalene | 3,6-metNAP | 252              | 253 (22) 250 (18)     |
| 32       | 9,10-dimethylnaphthalene | 9,10-metNAP | 252              | 253 (22) 250 (18)     |
| 33       | 1-methylpyrene | 1-metPY    | 252              | 253 (22) 250 (18)     |
| 34       | Retene       | RET         | 252              | 253 (22) 250 (18)     |
| 35       | 6-methylchrysene | 6-metCHR   | 252              | 253 (22) 250 (18)     |
| 36       | Dibenzo[b]fluoranthene | 2,3-metDBT | 252              | 253 (22) 250 (18)     |
| 37       | 1-methylbenzothiophene | 1-metDBT  | 252              | 253 (22) 250 (18)     |
| 38       | 3-methylbenzothiophene | 3-metDBT  | 252              | 253 (22) 250 (18)     |
| 39       | 2,3-dimethylbenzothiophene | 2,3-metDBT | 252              | 253 (22) 250 (18)     |
| 40       | 2,6-dimethylbenzothiophene | 2,6-metDBT | 252              | 253 (22) 250 (18)     |
| 41       | 2,4,6-trimethylbenzothiophene | 2,4,6-metDBT | 252              | 253 (22) 250 (18)     |
| 42       | Indole       | IND         | 252              | 253 (22) 250 (18)     |
| 43       | Carbazole    | CAR         | 252              | 253 (22) 250 (18)     |
| 44       | Acridine     | ACR         | 252              | 253 (22) 250 (18)     |
| 45       | Phenanthridine | PHR       | 252              | 253 (22) 250 (18)     |

* Based on mass spectra from authentic standards, compounds not present in NIST library.
XAD resin was transferred to an elution column and extracted with acetone and DCM. Both DCM fractions (eluent and ASE extract) were back-extracted using a 3% sodium chloride solution, dried using sodium sulphate, combined and then concentrated to 3 mL. The extracts were sent for further processing at the Air Quality Research Division (AQRD) ECCC laboratory in Ottawa, Ontario, where they were fractionated on silica gel solid phase columns with hexane and benzene, following AQRD method 3.03/5.1/M [46]. Further details on snowpack samples processing are provided in the supporting information. The benzene fraction (fraction 2) was analysed by GC×GC/ToF-MS using two column configurations. Sampling sites designated as AR6 (57.018 N; −111.485 W) and AMS5 (56.969 N; −111.482 W), located near the major developments, were used in this study.

2.3. GC×GC/ToF-MS analysis

Standard solutions and snowpack samples were analysed using a GC×GC/ToF-MS Pegasus 4D (Leco, St Joseph, MI, USA). The instrument consisted of an Agilent 7890B gas chromatograph (Palo Alto, CA, USA) equipped with a secondary oven, split/splitless injector and a consumable-free modulator operated at −80°C. The first column configuration used consisted of a liquid crystalline LC-50 (10 m × 0.18 mm × 0.10 µm) (J&K Scientific, Edwardsville, Nova Scotia, Canada) followed by a nano-stationary phase NSP-35 (1 m × 0.15 mm × 0.10 µm) (J&K Scientific). The second column configuration consisted on a high-polarity ionic-liquid SLB-IL60 (20 m × 0.18 mm × 0.07 µm) (Sigma-Aldrich, St. Louis, MO, USA) followed by a mid-polar Rxi-17 (1 m × 0.10 mm × 0.10 µm) (Restek, Bellefonte, PA, USA). The two GC columns in each set were connected using an Agilent CPM union (part No. G3182-61580). The data processing and analysis was performed using ChromaToF v.4.50.8. Optimised parameters for the column configurations are shown in Table 2. For comparison purposes, a more traditional column

| Parameter                      | LC-50×NSP-35 | SLB-IL60×Rxi-17 |
|--------------------------------|--------------|-----------------|
| Injection volume               | 1 µL         | 1 µL            |
| 1D column                      | LC-50 (10 m × 0.18 mm × 0.10 µm) | SLB-IL60 (20 m × 0.18 mm × 0.07 µm) |
| 2D column                      | NSP-35 (1 m × 0.15 mm × 0.10 µm) | Rxi-17 (1 m × 0.10 mm × 0.10 µm) |
| Total run time                 | 72 min       | 60 min          |
| Carrier gas                    | He           | He              |
| Inlet mode                     | Splitless    | Splitless       |
| Flow                           | 0.8 mL min⁻¹ | 1 mL min⁻¹      |
| Inlet temp.                    | 300°C        | 300°C           |
| 1D oven                        | 90°C (2 min), 20°C min⁻¹ to 170°C, 2°C min⁻¹ to 270°C (16 min) | 60°C (1 min), 5°C min⁻¹ to 300°C (11 min) |
| 2D oven                        | 120°C (2 min), 20°C min⁻¹ to 200°C, 2.5°C min⁻¹ to 325°C (16 min) | 80°C (1 min), 5°C min⁻¹ to 320°C (11 min) |
| Modulator temp. Offset         | 15°C         | 10°C            |
| Modulation period              | 4 s          | 4 s             |
| Hot pulse                      | 1.2 s        | 1.2 s           |
| Cool time                      | 0.8 s        | 0.8 s           |
| Transfer line temp             | 260°C        | 300°C           |
| Mass range                     | 50–350 u     | 50–350 u        |
| Acquisition rate               | 200 spectra s⁻¹ | 200 spectra s⁻¹ |
| Ion source temp                | 250°C        | 250°C           |
| Chiller temp                   | −80°C        | −80°C           |
configuration was also used in some sections of this study (non-polar × polar: Rtx-5 ms×Rxi-17). The optimised parameters for this last configuration were taken from previous publications [41,42].

2.4. Evaluation of 2D-separation

Standard solutions containing 45 PACs at concentrations ranging from 0.5 to 500 pg µL⁻¹ were used to evaluate both column sets. Retention times were determined using the highest modulated peak for each compound. To reduce variability due to peak broadening, the total peak area considered was limited to the sum of the three highest modulated peaks for each PAC, following a partial summation method [47]. Instrument detection limits (IDLs) for all 45 PACs were established as the lowest concentration at which GC×GC/ToF-MS analysis of the qualitative and quantitative ion of each PAC had signal-to-noise (S/N) ratio greater than 3:1.

The two-dimensional chromatographic resolution (R²D) for adjacent PACs was calculated using the 2D-resolution proposed by Giddings [48], based on the Euclidean norm of the resolution over the two dimensions and following equations (1) and (2):

\[
R_i = \frac{(t_{R(x+1)} - t_{R(x)})}{\frac{1}{2}(w_{(x+1)} + w_{(x)})}, \quad i = 1, 2, 
\]

\[
R_{2D} = \sqrt{R_1^2 + R_2^2}, 
\]

where \( R_i \) is the resolution in each dimension (\( i = 1, 2 \)), \( t_{R} \) the retention time for adjacent compounds (\( x \)) and (\( x + 1 \)), \( w \) is the peak width equivalent to four or five modulation periods in the first dimension (\( i = 1 \)), or to the full width at half height of the highest modulated peak for the second dimension (\( i = 2 \)) for the same pair of compounds (\( x \) and \( x + 1 \)).

First dimension retention indexes (RIs) were determined as the average of eight consecutive injections (\( n = 8 \)) of the standard solution at 50 pg µL⁻¹ following equation (3):

\[
RI = RI_{std} + \left[ \frac{1}{t_{R_{target}} - 1{t_{R_{std}}}} \left( 1{t_{R_{target}}} - 1{t_{R_{std}}} \right) \right] \times 100, \]

where \( 1{t_{R_{std}}} \), \( 1{t_{R_{(std+1)}}} \), \( 1{t_{R_{target}}} \) are the retention times in the first dimension for the preceding, subsequent bracketing compounds, and for the target compound. \( RI_{std} \) is the fixed index for the preceding bracketing compound. For compounds eluting before or after the bracketing compounds, the \( RI_{std} \) used was either the first or last bracketing compound, respectively. The bracketing compounds and fixed \( RI_{std} \) used in SLB-IL60×Rxi-17 were naphthalene (\( RI_{std} = 200 \)), phenanthrene (\( RI_{std} = 300 \)), chrysene (\( RI_{std} = 400 \)), benzo[a]pyrene (\( RI_{std} = 450 \)) and benzo[g,h,i]perylene (\( RI_{std} = 500 \)). In LC-50xNSP-35 naphthalene was replaced with fluorene (\( RI_{std} = 200 \)) as the lowest bracketing compound, because of weak interactions between the stationary phase and naphthalene, which eluted within the solvent.

Snowpack samples collected near the major industrial developments in the oil sands area were used to evaluate the 2D-space occupation following a conditional entropy
approach \[49\]. Initially, the data processing software was arbitrarily set to identify 2000 peaks in each sample. All peaks were then classified based on library similarity (NIST 2011 and Wiley v.9) and S/N, and only those peaks with similarities >70% and S/N > 50:1 were used. Normalised retention times (from 0 to 1) in both dimensions were used to create a 11 x 11 matrix (based on Sturges method for \(~\sim\)1400 data points) that represented the number of peaks eluting within each time interval in both dimensions. The resulting matrices were used to determine the conditional entropy and orthogonality for each configuration, expressed as a percentage that represents the occupation of the two-dimensional space \[49\].

3. Results and discussion

3.1. LC-50×NSP-35

Repeatability parameters (measured as relative standard deviations ‘RSD’) for LC-50×NSP-35 (\(n = 8\)) showed good stability for retention times in the first dimension (1\(t_R\)) (average: 0.3%, range: 0–0.9%), retention times in the second dimension (2\(t_R\)) (average: 0.7%, range: 0.4–1.5%), retention indexes (RI) (average: 0.2%, range: 0–1.5%), peak areas (average: 4.3%, range: 0.4–25%) and peak heights (average: 2.7%, range: 0.7–6.3%) (Table S1). Since the retention times assigned are based on the highest modulated peak, most of the variability in RIs and 1\(t_R\) was caused by differences in the distribution of modulated peaks, fluctuating between in-phase and out-of-phase \[47\]. The average \(R^2_D\) for adjacent PACs was 7.45 (ranged 0–44) (Table S2), with some co-elutions observed in the first portion of the chromatogram. The average IDL for 45 PACs was 2.5 pg \(\mu\)L\(^{-1}\) (range 0.5 to 10 pg \(\mu\)L\(^{-1}\)) (Table S2).

Although the average \(R^2_D\) was relatively high (7.45), and some well-known co-elution cases were resolved (i.e. chrysene, triphenylene and benzo[\(a\)]anthracene; benzo[\(k\)] and benzo[\(b\)]fluoranthene; benzo[\(e\)] and benzo[\(a\)]pyrene), the quality of the chromatographic separation was not uniform throughout the run. Low molecular weight PACs (<160 u) (e.g. naphthalenes, alkylated naphthalenes, indole, acenaphthylene and acenaphthene), showed weak interactions with the stationary phase and eluted in the early section of the chromatogram (<5 min, 7% of total run time) and some with the solvent (Table S2). Peak tailing and co-elutions were observed in this section, and they affected the determination of IDLs and peak areas which showed to be relatively high particularly for dimethylnaphthalenes (10 pg \(\mu\)L\(^{-1}\), \~16% peak areas RSDs) (Table S2). Mid-molecular weight PACs (160–260 u) occupied most of the 2D-chromatogram and eluted between 5 and 50 min (63% of the total run time). In general, good peak shapes were observed in this section, with 3 to 4 modulated peaks per compound and no cases of co-elutions. Relatively low IDLs were determined for compounds eluting in this section (0.5 to 1 pg \(\mu\)L\(^{-1}\)) except for dibenzothiophene and benzo[\(b\)]fluorene (10 pg \(\mu\)L\(^{-1}\)) (Table S2). Dibenzothiophene also showed relatively high variability for total peak area (12%) and peak height (6%) (Table S1). Higher molecular weight PACs (>260 u) showed strong interactions with the stationary phase and eluted in the last portion of the chromatogram (>50 min, \~31% of the total run time). The number of modulated peaks increased (>4) due to peak broadening in the first dimension, and therefore IDLs and repeatability parameters for peak area and peak height (10 pg \(\mu\)L\(^{-1}\) and \>20%) were affected.
(Table S1 and S2). Longer modulation times in the last portion of the chromatogram can be used to improve these parameters without affecting the chromatographic separation due to the relatively high $R_{2D}$ (Table S2).

The 2D space occupation was evaluated using the aromatic fractions of two snowpack samples (AR6 and AMS5). All peaks corresponding to column bleed, solvent residues, peaks with library similarities below 70% (NIST 2011 and Wiley v.9) and S/N below 50:1 were excluded from the analysis. The number of tentatively identified peaks was therefore reduced from the initial arbitrary 2000 peaks to 1473 for sampling site AR6, and 1320 for sampling site AMS5, with a relatively high number of thia-arenes and aza-arenes in both cases (Table S3). The 2D-space occupation, based on a conditional entropy approach [49], was determined to be 86.3% (AR6) and 86.4% (AMS5). The overall elution pattern of ~1400 compounds did not follow the diagonal distribution commonly observed for PACs, and appeared to shift directions as the run progressed in time: as the $t_R^1$ increased, the $t_R^2$ increased, then decreased and then increased again (Figure 1). This shifting was caused by changes in the temperature programming of the first dimension column, and it has been observed at different modulation periods [41]. In general, similar elution patterns were observed for unPAHs, aPAHs, thia-arenes, aza-arenes in both snowpack samples, with weak molecular similarity grouping and thereby a more spread-out distribution (Figure 2).

An elution pattern described as a ‘roof-tile’ effect can be observed when a more traditional polar × non-polar column combination (e.g. Rtx-5 ms×Rxi-17) is used for the analysis of complex samples [18,45], and the same was observed for snowpack samples from the oil sands area of Alberta (Figure 2(a,b)). The roof-tile visual effect is produced when a group of compounds sharing similar physicochemical properties is positioned within a limited region in the 2D-space, and follow a diagonal distribution. This can be visually useful for molecular similarity grouping and initial screening, however the chromatographic separation of PACs within each group is limited (Figure 2(a,b)). The roof-tile effect in LC-50×NSP-35 was not significant, favouring the separation of isomeric compounds (Figure 2(c,d)).

![Figure 1. Distribution of ~1400 compounds tentatively identified (S/N > 50:1, library similarity >70%) in the aromatic fraction of samples AR6 and AMS5 using LC-50×NSP-35. Each dot represents the retention time of the highest modulated peak for each compound. Lighter dots are used to show where specific groups of PACs eluted in the 2D-chromatogram. All retention times were normalised from 0 to 1. See online version for coloured figures.](image-url)
Room temperature ionic liquids are a class of non-molecular ionic solvents with low melting points that have found several applications as stationary phases in GC [50,52–54]. The unique properties of ionic liquids (i.e. low volatility, high viscosity, good thermal stability, variable polarities and dual nature to interact with polar and non-polar compounds) make them good candidates for GC stationary phases [53]. SLB-IL60 is a relatively high-polarity stationary phase (higher than wax-phases in the polarity scale), and was used in the first dimension in GC×GC/ToF-MS. By combining SLB-IL60 in the first dimension with a mid-polarity Rxi-17 in the second, we focused on the small differences in molecular properties of thia-arenes and aza-arenes, in order to improve their separation from other matrix components, therefore improving their detectability.

Repeatability parameters for SLB-IL60×Rxi-17 (n = 8) also showed good stability for $t_R$ (average: 0.06%, range 0–0.2%), $2t_R$ (average: 0.9%, range: 0.2–4.3%), $RIs$ (average: 0.04%, range: 0–0.1%), peak areas (average: 17%, range: 1–43%) and peak heights (average:
15%, range: 1–40%) (Table S4). Similar to LC-50×NSP-35, the retention times assigned are based on the highest modulated peak, and therefore the variability in $R_t$ and $^1t_R$ can be caused by differences in the distribution of modulated peaks. Although peak shapes in SLB-IL60×Rxi-17 were consistent throughout the chromatogram, the largest variability in peak areas and peak heights was also observed in dimethylnaphthalenes (~10%), dibenzothiophene (>30%) and high molecular weight unPAHs (dibenzo[a,h]anthracene, picene and benzo[g,h,i]perylene, >20%) (Table S4). The average $R_{2D}$ for adjacent PACs was determined to be 7.05 (ranged 0–26), while the average IDL was 2.5 pg µL$^{-1}$ (range from 0.5 to 10 pg µL$^{-1}$) (Table S5). IDLs were relatively high (10 pg µL$^{-1}$) for dibenzothiophene, benzo[b]fluorene, perylene, dibenzo[a,h]anthracene, picene and benzo[g,h,i]perylene (Table S5).

Following the same procedure as with LC-50×NSP-35, the 2D-space occupation in the analysis of the aromatic fraction of two snowpack samples (AR6 and AMS5) was evaluated for SLB-IL60×Rxi-17. All peaks corresponding to column bleed, solvent residues, compounds with library similarities below 70% (NIST 2011 and Wiley v.9) and S/N below 50:1 were excluded. Therefore, the number of tentatively identified compounds was reduced from the initial arbitrary 2000 peaks to 1313 (AR6) and 943 (AMS5) (Table S3). The overall distribution of ~1300 compounds appeared to be similar to more traditional column combinations. However, a group of compounds, all of them tentatively identified as carbazoles, benzo[a]carbazoles, alkylated carbazoles and alkylated benzo[a]carbazoles, were resolved from the samples matrix and other PACs in the second dimension (Figure 3). The 2D-space occupation, based on conditional entropy [49], was determined to be 79.8% (AR6) and 85.8% (AMS5), and can be a consequence of the isolation of this group of aza-arenes. The distribution of isomeric groups of PACs in the 2D chromatographic space did not show a typical roof-tile effect (Figure 2(e,f)), compared to Rtx-5 ms×Rxi-17 (Figure 2(a,b)). However, molecular similarity grouping was more evident for some PAC groups if we compare it to LC-50×NSP-35 (Figure 2(c,d)). In general, SLB-IL60×Rxi-17 was observed to favour molecular similarity grouping over isomer...
separation, particularly for some aza-arenes. The separation of this group of aza-arenes from the sample matrix and other PACs can be potentially useful to increase their detectability in complex samples where these compounds are often present in low relative abundance.

3.3. PAC identification and quantitation problems solved by GC×GC/ToF-MS

There is widespread agreement that the addition of thia-arenes and aza-arenes to regular monitoring lists of PACs in the environment could be beneficial for toxicity and source apportionment studies [55]. This is particularly important for situations where fossil fuels and their derivatives are present [55]. However, several factors related to the complexity of the sample, such as sample matrix interferences, multiple isomeric compounds sharing similar mass fragmentation patterns, the lack of authentic standards to represent all target compounds and instrument limitations can contribute to an inaccurate identification and quantitation of thia-arenes, aza-arenes and other PACs in one-dimensional GC/MS.

GC/MS is routinely used in selected ion monitoring mode (SIM) to quantitate PACs in a variety of environmental samples [35]. However, it has been shown that GC/MS can produce inaccurate and imprecise concentration estimates due to incorrect homologue peak assignments [10,25,37]. In SIM mode, compound identification is based solely on retention times of a specific mass fragment, and therefore common ion effects from the matrix can result in overestimations of target compounds [37]. Moreover, relying on a single fragmentation pattern is insufficient to capture all possible isomers whether run under full scan or SIM conditions [37].

To illustrate this, snowpack sample AR6 was analysed by GC/MS following ECCC method 3.03/5.1/M, and using a Rtx-5 ms GC column [46]. We focused particularly on two mass fragments commonly used for the identification of C4-Phenanthrenes/Anthracenes (C4-PH/AN) (m/z 219 and m/z 234). Approximately 13 peaks can be observed in the section of the chromatogram were C4-PH/ANs are expected to elute, if only fragments m/z 219 and m/z 234 are considered (Figure 4(a)). Many of these peaks could be initially identified as one of the two fragmentation patterns for C4-PH/ANs (Figure S1). However, if other mass fragments are included in the chromatogram (e.g. m/z 222, a major ion fragment in some thia-arenes) (Figure 4(b)), accurate peak assignment becomes challenging. GC×GC/ToF-MS can provide the chromatographic separation power needed to resolve some of these problems, even when using traditional column sets (i.e. Rtx-5 ms×Rxi-17) [41] (Figure 4(c)). Due to the chromatographic separation in the second dimension, it was determined that the peaks that contained fragment m/z 222 and were previously believed to be C4-PH/ANs, can be tentatively identified as thia-arenes: C1-phenanthro[4,5-b,c,d]thiophenes (>80% similarity) and phenanthro[4,3-b]thiophene (>65% similarity) (Figure 4(c)). These thia-arenes share similar mass fragmentation patterns with C4-PH/ANs, that include fragments m/z 219 and m/z 234. Although m/z 219 is not the most abundant mass fragment in C1-phenanthro[4,5-b,c,d]thiophenes (Figure S1), this thia-arene is present in high enough relative abundance that it can affect the quantitation of C4-PH/ANs. Therefore, overestimation errors in the analysis of C4-PH/ANs in samples containing high relative abundance of thia-arenes (such as samples form the Athabasca oil sands area) are possible. These kind of interferences
have been previously described, and an additional fractionation process during sample preparation was suggested as a possible solution [24]. The use of LC-50×NSP-35 improved the chromatographic separation of isomers, and further reduced the risks of false positives and overestimations (Figure 4(d)), and therefore can be an alternative for the analysis of thia-arenes in complex samples.

Another advantage of using GC×GC/ToF-MS equipped with non-traditional column configurations, such as LC-50×NSP-35 and SLB-IL60×Rxi-17, is the production of cleaner chromatograms and mass spectra, which can help in the positive identification of compounds with low relative abundance. This was observed in the analysis of snowpack samples, particularly in sections where aza-arenes and aPAHs eluted at similar retention times when using Rtx-5ms×Rxi-17 (Figure 5(a)). In this particular case, C2-benzo[a]anthracenes/chrysene/triphenylenes (C2-BCT) and aza-arenes identified as dibenzo[b,def]carbazole (85% similarity), dibenzo[a,c]phenazine (49% similarity) and 2,6-diphenylpyridine (78% similarity) were resolved in the second dimension only (Figure 5(a)). Although, the PACs described did not share the same fragmentation pattern, and therefore the software deconvolution tool can be used for an accurate identification, the similarities with the NIST library were still relatively low (Figures S2–S4). The LC-50×NSP-35 and SLB-IL60×Rxi-17 column sets improved the overall chromatographic separation, and the isolation of targeted compounds from the sample matrix. The NIST library similarities increased as a consequence of a cleaner mass spectra (dibenzo[b,def]carbazole: 90%, dibenzo[a,c]phenazine: 98%, 2,6-diphenylpyridine: 87% for LC-50×NSP-35; and dibenzo[b,def]
carbazole: <50%, dibenzo[a,c]phenazine: 96%, 2,6-diphenylpyridine: 88% for SLB-IL60×Rxi-17) (Figure 5(b,c)). The relatively low similarity for dibenzo[b,def]carbazole when SLB-IL60×Rxi-17 was used was a consequence of a co-elution with other aza-arenes in the region where most aza-arenes are isolated (Figure 3).
4. Conclusions

GC×GC/ToF-MS, equipped with LC-50×NSP-35 and SLB-IL-60×Rxi-17 column sets, was used for the analysis of standard mixtures of 45 PACs and snowpack samples collected near the major industrial developments in the Athabasca oil sands area, in Canada. Both configurations showed good stability for retention times and RIs (n = 8). Total peak area and peak height variability was on average 4% and 16%, respectively, with relatively high variability (>20%) observed for late eluting unPAHs (BghiP and PIC), and for DBT (>30%) particularly with SLB-IL60×Rxi-17.

In general, LC-50×NSP-35 favoured the separation of isomeric compounds. It did not show a significant roof-tile effect (compared to more traditional Rtx-5 ms×Rxi-17), and showed overall weak molecular similarity grouping of PACs. The 2D space occupation in the analysis of complex samples containing ~1400 tentatively identified peaks was >80%. This configuration can be considered as an alternative for the analysis of thia-arenes in complex environmental samples. It showed enhanced separation of compounds with similar mass fragmentation patterns without any extra sample preparation or fractionation process. However, the quality of the separation was not uniform throughout the run, especially for early eluting PACs (MW<160 u).

Alternatively, SLB-IL60×Rxi-17 favoured molecular similarity grouping over isomer separation. This was particularly evident for carbazoles, benzo[a]carbazoles and their alkylated derivatives which were isolated from the sample matrix and other PACs. This can be important for the analysis of aza-arenes in complex samples, due to their relatively low abundance compared to other PACs.

In addition, the use of a GC×GC/ToF-MS provided evidence of potential errors in the identification and quantitation of aPAHs in complex samples obtained from the oil sands area of Alberta, and also containing thia-arenes and aza-arenes. LC-50×NSP-35 and SLB-IL-60×Rxi-17 were both used to improve and increase the resolution of PACs, producing cleaner mass spectra and preventing potential false positives and overestimations.

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Disclosure statement

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