High Performance Thin Layer Chromatography (HPTLC) Coupled with DESI-Ion Mobility-Mass Spectrometry: A Novel Approach for Petroleum Characterisation

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INTRODUCTION

The complexity of petroleum samples places high demands on analytical chemists; however, the implementation of petroleomic methodologies has enabled a greater understanding of petroleum at a molecular level.[1] The ability to fully characterize the molecular composition of petroleum samples provides significant insight into the likely value of that petroleum. To that end, the ability to utilize a "tool-box" approach for comprehensive characterization is highly advantageous.

Recently, TLC with femtosecond-laser ablation-ICP MS has been used to analyse vanadium and nickel components of asphaltenes,[2] and TLC with laser desorption ionisation-FTICR MS was employed in the analysis of crude oil, among other samples.[3] Furthermore, DESI has been shown to be applicable to the analysis of components of lubricant oil;[4] while there is increasing interest in the use of ion mobility for petroleomics studies.[5,6]

In this work, we consider the efficacy of analyzing petroleum samples using HPTLC separation with DESI coupled to ion mobility -mass spectrometry (IM-MS) as a complementary characterization approach. DESI enables the direct analysis of samples under ambient conditions, while ion mobility offers the potential for isomer deconvolution thus affording more comprehensive characterization than mass resolution alone.

METHODS

Data Acquisition and Processing:

HPTLC data were acquired and visualized using winCATS software (CAMAG)

Mass Spectral data were acquired using MassLynx v4.1 (Waters Corporation) and processed using MassLynx v4.1, DriftScope v2.8, High Definition Imaging (HDI) software v1.4 (Waters Corporation), and PetroOrg S-10.2 (Omics LLC)[7]

Samples:

Stock solutions of samples were prepared at 25 µg/µL in toluene and spotted on the HPTLC plate (*Figure 1)*

- Crude oil 1
- Crude oil 2
- Vacuum residue: heated to 110 °C before HPTLC analysis
- Atmospheric residue: heated to 120 °C before HPTLC analysis
- Reference bitumen: heated to 110 °C (<30 min.) before HPTLC analysis

A reference standard was prepared at 1 µg/µL in toluene

- Vanadyl porphyrin

HPTLC Plate:

HPTLC PHASE:

- Silica gel 60 Diol F254S (Merck)

ELUTION SOLVENTS:

- Heptane/Toluene (65/35 v/v)

Figure 1. Samples and reference standards separated by HPTLC

HPTLC methodology:

- 0.4 µL (10 µg) and 2 µL (50 µg) of each sample and the reference bitumen were automatically spotted using an ATS 4 device (CAMAG)
- 1 µL (1 µg) of vanadyl porphyrin standard was automatically spotted using the ATS 4
- Sample migration was carried out using an AMD2 device (CAMAG) until the heptane/toluene solvent front reached 39 mm
- The HPTLC plate was visualized using a TLC Visualizer (CAMAG) at UV wavelengths of 254 nm and 366 nm
- UV absorbance chromatograms were obtained using a TLC Scanner 4 (CAMAG) UV densitometer at 254 nm

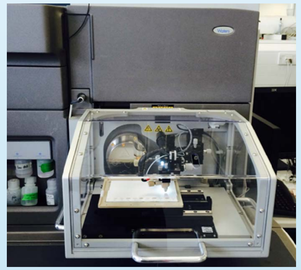

Figure 2. The DESI source installed on the SYNAPT G2-Si HDMS instrument

SYNAPT G2-Si HDMS conditions:

A Desorption ElectroSpray Ionization (DESI) source (Prosolia) was installed on a SYNAPT G2-Si HDMS instrument (Waters Corporation) *(Figure 2)*

DESI and ion mobility-MS conditions were optimized as follows:

- Ionization mode : DESI+
- Capillary voltage : 3.5 kV
- Cone voltage: 80 V
- Source temperature: 120 °C
- IMS Wave velocity: 1000 m/s (fixed)
- IMS Wave height: 40 V
- IMS cell pressure: 3.3 mbar
- DESI spray solution: 98:2 MeOH:H_2O v/v + 1 ng/µL Leucine Enkephalin
- DESI spray solution flow rate: 3 µL/min
- DESI gas pressure: 6 bar

RESULTS AND DISCUSSION

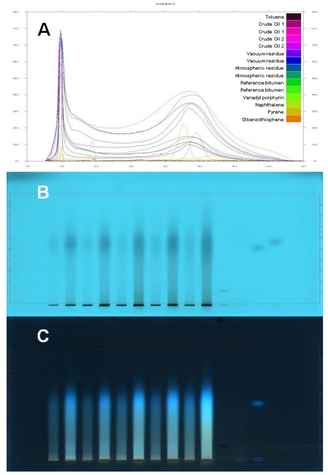

Figure 3. [A] Example UV absorbance chromatograms for each sample, and reference compounds;
[B] Example UV visualization of silica gel 60 Diol F254S HPTLC plate at wavelength 254 nm;
[C] Example UV visualization of silica gel 60 Diol F254S HPTLC plate at wavelength 366 nm

Figure 3 shows example results from UV analyses of HPTLC separations. Four additional standard compounds (toluene, naphthalene, pyrene, and dibenzothiophene) were spotted on the original TLC plate. These four standard compounds were removed when the plate was cut to fit on the DESI platform.

DESI parameters were optimized to maximize extraction of ions from the plate surface and transmission into the instrument. Both MeOH:H_2O and MeOH:Toluene:IPA were evaluated as potential spray solvents. For this initial work, MeOH:H_2O was chosen and Leucine Enkephalin was spiked into the solution at a concentration of 1 ng/µL for internal mass calibration (LockMass: *m/z* 556.2766).

Sprayer and extraction capillary positions were optimized relative to each other with maximum ion extraction and transmission observed at ~2 mm above plate surface, ~7 mm gap between sprayer and capillary orifice, 76° impact angle between sprayer and plate surface, and ~10° angle between collection capillary and plate surface.

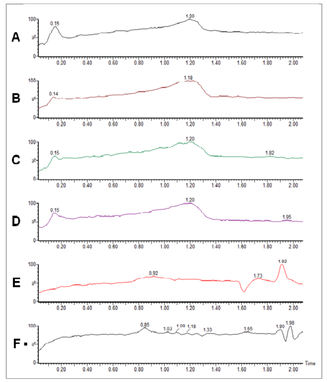

*Figure 4. Total Ion Chromatograms (TICs) for DESI acquisitions along the length of each HPTLC separated sample. **A**: 50 µg Crude Oil 1; **B**: 50 µg Crude Oil 2; **C**: 50 µg Vacuum residue; **D**: 50 µg Atmospheric residue; **E**: 50 µg Reference bitumen; **F**: 10 µg Vanadyl porphyrin*

Data were initially reviewed in DriftScope and MassLynx software. Figure 4 show the TICs for each sample. The profiles can be seen to broadly resemble the information observed in the UV visualization and chromatograms in Figure 3.

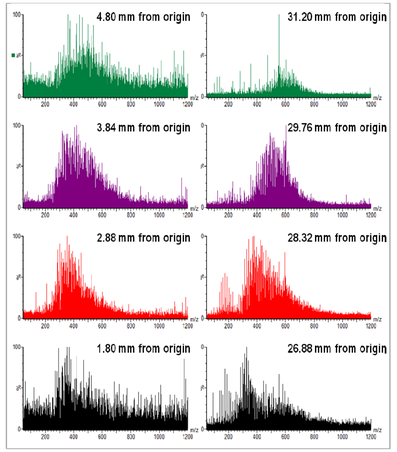

Figure 5. Background subtracted spectra for Sample B (50 µg Crude Oil 2) at progressive distances from the sample spot origin.

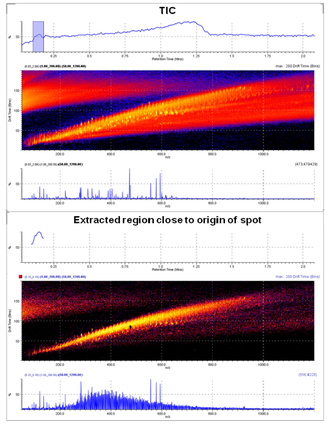

Figure 6. Data for 50 µg Crude Oil 2 (sample B) viewed in DriftScope software. Upper images: Total Ion Chromatogram (TIC), mobilogram, and combined spectrum across the whole acquisition. Lower images: extracted region close to sample spot origin, extracted mobilogram, and associated extracted mass spectrum

Background subtracted spectra were then generated from different points across the TIC for sample B (Crude oil 2). These spectra are shown in Figure 5. Figure 6 illustrates data for sample B viewed in DriftScope. The TIC is dominated by background ions, but information from the UV visualization of the TLC plate helped to highlight key regions of interest, and the petroleum component is clearly visible when a portion of the data is extracted. The spatial distribution of individual ions of interest can be imaged along the HPTLC separation, as shown in Figure 7. By using this approach, we observed that even mass ions were found closer to the spot origin while odd mass ions occurred closer to the solvent front; hence we could deduce that even mass ions were more polar and odd mass ions were more non-polar.

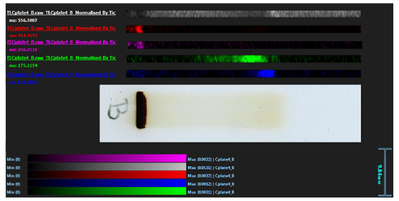

Figure 7. HDI DESI imaging of the spatial distribution of representative ions from sample B. White: m/z 556.300 (LockMass); Red: m/z 354.3552; Pink: m/z 256.2531; Green: m/z 175.1154; Blue m/z 629.5800

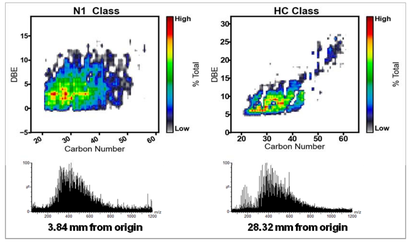

Figure 8. PetroOrg software images for N1 class that was localized near the spot origin and the HC class that was distributed closer to the solvent front

By using PetroOrg software, ion mobility data can be further processed to reveal compositional information about the sample. This provided confirmation that the more polar nitrogen-containing species are localized near the origin and the more non-polar hydrocarbon class is found nearer the solvent front.

CONCLUSION

- **DESI-IM-MS was successfully employed for the analysis of petroleum samples separated by HPTLC**
- **HPTLC separation of key classes of compounds made data review and processing significantly easier since spectra from different regions of the TLC plate could be processed individually**
- **This multidimensional approach offers great potential for detailed studies of highly complex petroleum samples**
- **Further work will include investigation of additional DESI spray solvent systems to enhance extraction of less polar sample components, and comparisons between the separations achieved using different HPTLC phases and different elution solvents**

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