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I have over 20 years experience in the analysis of crude oils. After achieving a 1st Class B.Sc. Hons in Environmental Science from the University of Plymouth and obtained a Ph.D. in 2000 characterising chemical fractions from sediments and bacteria. In 2003 I completed a 3-year NERC Post-doc, investigating the molecular characterisation of chromatographically 'unresolved complex mixtures (UCMs) obtained from crude oils using preparative-gas chromatography. Between 2000-2008 I was employed as a Scientific Officer in the School of Earth, Ocean and Environmental Sciences and developed our high temperature gas chromatography capability for use in wax analysis in crude oils and have been offering this service commercially since 2007. In 2008 I was seconded to a two-year Joint Industry Project on behalf of five oil companies and developed a method for the quantification of C<sub>80</sub> tetraacids 'ARN' acids in crude oils with sample preparation using ion exchange solid phase extraction, which is still being used for commercial analysis. In 2011 my role changed to a Senior Research Fellow and later, in 2019, to a Technical Specialist. My current interests include developing chromatographic separation techniques for organic geochemistry applications, especially isolation of high molecular weight organic compounds and the application of high temperature gas chromatography with flame ionisation detection (HTGC-FID) and HTGC coupled with mass spectrometry.



- A. Class-type separation of petroleum using ion exchange solid phase extraction and analysis of amenable fractions using high temperature gas chromatography with flame ionisation detection.
- B. Wax analysis using high temperature gas chromatography with flame ionisation detection.

### Method description in brief.

This proposal relates to two methods of sample preparation: (A) Isolation of chemical classes using ion exchange solid phase extraction, (B) preparation of samples for 'wax' analysis. Amenable fractions obtained in (A) and samples prepared in (B) will be analysed using high temperature gas chromatography with flame ionisation detection (HTGC-FID).

- A. Chemical class fractionation: This proposal envisages gravimetric determination of compound classes obtained by sequential ion exchange chromatography (Robson et al. 2017 Class type separation of the polar and apolar components of petroleum. *Analytical Chemistry* **89**, 2919-2927) and analysis of amenable fractions (sulfoxides, sulfones, carbazoles, methylated naphthenic acids, saturates, aromatics, fluorenones, xanthenes and thiophenes) using HTGC-FID. Aliquots of each sample will be subjected to extended nitrogen blow-down to account for potential losses of volatiles. Solubilised samples will be separated using strong cation exchange (SCX) solid phase extraction (SPE) to obtain 'cationic' fractions ('asphaltene', sulfoxides, unknown fraction, and quinolines). Unretained material from SCX separation will undergo strong anion exchange (SAX) SPE to obtain 'anionic' fractions (sulfones, carbazoles and naphthenic acids). Unretained material from SAX separation will be separated using adsorption SPE chromatography on silica to obtain 'non-ionic' fractions (saturates, aromatics, fluorenones, xanthenes and thiophenes). Amenable fractions (SCX sulfoxides; SAX sulfones, carbazoles and methyl esterified naphthenic acids; Si all fractions) will be analysed using HTGC-FID. All fractions can be made available for confirmatory mass spectral analysis by another laboratory if required.
- B. Wax analysis: For wax samples and analysis of waxes, a separate approach will be used: an aliquot of each sample (WHOLE) and its corresponding 'topped' fraction (TOPPED) prepared using a proprietary method (T-SEP; <http://www.kat-lab.com/t-sep%C2%AE.html>) and diluted for HTGC-FID analysis using a 'hot' injection technique. The *n*-alkanes in each fraction are integrated and the data merged to provide a carbon number weight percentage profile of the *n*-alkanes (this can be modified to integrate carbon number range).

HTGC-FID analysis: HTGC-FID will be carried out using an Agilent 8890 gas chromatograph with cool-on-column inlet (0.5 µL injection; track oven mode, +3°C), VF-5ht (15m x 0.25mm x 0.1µm) Ultimetall column and high temperature FID jet (435°C). Peak identifications will be made by retention time matching for linear alkanes (C<sub>10-30,40,50,60</sub>; even carbon numbered *n*-alkanes ca. C<sub>20-106</sub>) and linear fatty acid methyl esters (*n*FAMES; C<sub>8-20</sub>; even carbon numbered ca. C<sub>20-80</sub>). Semi-quantitative analysis of *n*-alkanes will be carried out using an external *n*-alkane standard (C<sub>10-30,40,50,60</sub>). Different approaches can be applied to the semi-quantitation of amenable fractions obtained from the ion exchange separations. For example, a carbon number fraction approach (total peak area from front of one *n*-alkane to the front of the next carbon number *n*-alkane, including any area due to the presence of unresolved complex mixture

### Applicability of method.

- A. Chemical class fractionation: This method has wide applicability to the chemical class separation of petroleum and many of its derivatives. It has been used previously to investigate the composition of crude oils differing in API gravity (12.1-38.3°), TAN (0.1-3.6 mg KOH), sulphur (0.25-2.70%) and asphaltene (0-11%) content, thermal maturity (immature to late mature) and biodegradation (0.9-8.2 and not present pristane/<sub>n</sub>C<sub>17</sub> ratio).
- B. Wax analysis: Wax analysis has been carried out on a wide range of petroleum samples including condensates, black crude oils (including high melting point waxy crudes) and deposits.

HTGC-FID analysis: Most of the fractions obtained in the chemical class fractionation are amenable to GC. The carbon number range for the HTGC-FID set-up proposed herein is approximately C<sub>8-106</sub> for *n*-alkanes (114-1486 Da), C<sub>8-80+</sub> *n*-FAMES (176 -1328 Da), so-called 'Arn' C<sub>80-8:0-8</sub> tetraacids (*per*-methyl esterified; 1301-1351 Da), trimethylsilylated (even numbered) *n*-alcohols up to ca. C<sub>80</sub> (1211 Da), trimethylsilylated alkylethoxylates. LOD Ca. 20 pg on column for C<sub>87</sub>H<sub>176</sub> hydrocarbon.

### Sample preparation required.

- A. Chemical class fractionation: Transfer around 200 mg of sample dissolved in tetrahydrofuran/0.1% formic acid (THF/FA; 2 mL in 3 aliquots; mix by vortex, 10s) onto pre-conditioned (5 mL, THF/0.1% FA) strong cation exchange (SCX) solid phase extraction (SPE) cartridge (500 mg/3 mL) and sequentially elute with THF/0.1%FA (5 mL; SCX0 unretained material), toluene (5 mL; SCX1 'asphaltenes'), THF (5 mL; SCX2 'sulfoxides'), THF/0.1% water (H<sub>2</sub>O)/0.2% ammonia (NH<sub>3</sub>) (5 mL; uncharacterised), THF/0.1% H<sub>2</sub>O/0.5% NH<sub>3</sub> (5 mL; 'quinolines'), flush dried (20s) between eluents. Remove solvent from all fractions under a gentle stream of nitrogen (N<sub>2</sub>). Reconstitute SCX0 in diethyl ether (DEE)/0.1% H<sub>2</sub>O/0.1% NH<sub>3</sub> and transfer (2 mL in 3 aliquots, mix by vortex, 10s) onto pre-conditioned (2% NH<sub>3(aq)</sub>) (5 mL), H<sub>2</sub>O (5 mL), DEE/0.1% H<sub>2</sub>O/0.1% NH<sub>3</sub> (5 mL)) strong anion exchange (SAX) SPE cartridge (500 mg/3 mL) and elute sequentially with DEE/0.1% H<sub>2</sub>O/0.1% NH<sub>3</sub> (5 mL; SAX0 unretained material), toluene (5 mL; SAX1 'sulfones'), THF (5 mL; SAX2 'carbazoles') and THF/0.1% H<sub>2</sub>O/2% FA (5 mL; SAX3 'naphthenic acids'), flush dried (20s) between eluents. Remove solvent from all fractions under a gentle stream of nitrogen (N<sub>2</sub>). Reconstitute SAX0 in *n*-hexane and transfer onto pre-conditioned (THF, 5 mL; dichloromethane (DCM), 5 mL; *n*-hexane, 5 mL) silica SPE cartridge (200 mg, 3 mL) and elute sequentially with *n*-hexane (5 mL; S0 'saturates'), 20% DCM/*n*-hexane (5 mL; S1 'aromatics'), 50% DCM/*n*-hexane (5 mL; S2 'fluorenones'), DCM (5 mL; S3 'xanthenes') and THF (5 mL; S4 'thiophenes'), flush dried (20s) between eluents. Remove solvent from all fractions under a gentle stream of nitrogen (N<sub>2</sub>). Weigh all extracts. Amenable fractions will be diluted to an appropriate concentration for HTGC-FID analysis.
- B. Wax analysis: Liquid, semi-liquid (waxy oils) and solid (oily waxes) samples can be prepared using the T-SEP method. A sample of WHOLE oil and a TOPPED sample with non-wax material removed are prepared for analysis using HTGC-FID and semi-quantified using external *n*-alkane standards.

### Method strengths.

- A. Chemical class fractionation: This method is non-specialised and can be performed in all laboratories, requiring only a vacuum manifold. It provides gravimetric determination of compound classes and therefore would be useful for 'read-across' studies. HTGC-FID is relatively simple, cheap and can be used for the analysis of most of the petroleum sub-fractions. HTGC-FID is good for quantitation with good linear response and does not suffer from some of the issues encountered in mass spectral studies (e.g., ion suppression, differences in response factors).
- B. Wax analysis: HTGC-FID is the method of choice for semi-quantitative wax analysis. Inclusion of a topping procedure significantly increases the sensitivity for higher carbon number compounds by removing non-wax components such that additional *n*-alkanes can be detected compared to the whole oil.

### Estimated time for analysis.

The estimated time for sample preparation would be about two days with sample analysis taking around twelve days using manual injection. Around five days would be envisaged for data processing, interpretation, and report preparation. The total time would be about four working weeks (part time).

### Method weaknesses.

- A. Chemical class fractionation: Some fractions obtained in this manner are not amenable to HTGC-FID. Analysis using HTGC-FID provides no molecular information about constituents other than a comparative idea of modified boiling point distribution relative to external standards. A procedural blank is required to account for compounds that may be extracted from the SPE media. There is likely to be a lack of standards for individual  $>C_{30}$  compounds, which would make assignment even by retention time matching difficult. Despite the high efficiency of gas chromatographic separations, there is likely to be a lack of separation capacity as the number of potential isomers increases with carbon number, making the appearance of UCMs more likely. Whilst GCxGC separations greatly improve the capability of resolving UCM components it has not yet been demonstrated for separations above  $350^{\circ}\text{C}$ . Our HTGC coupled with Select eV™ (tunable 12-70eV) time-of-flight mass spectrometry system offers some degree of molecular characterisation and may form part of a more extensive subsequent investigation.
- B. Wax analysis: Analysis using HTGC-FID provides no molecular information about constituents, although *n*-alkane assignment based on retention time matching with external standards along with regular homology of *n*-alkanes is widely accepted.

### Result interpretation / visualisation / presentation.

- A. Chemical class fractionation: Gravimetric data (weight %) will be determined for chemical classes obtained through ion exchange SPE. This will identify which fractions are quantitatively important for each sample and identify differences between substrates. The fractionation of petroleum substrates based on chemical classes may be useful for future fraction-toxicity assessments to identify which chemical classes pose the highest toxicity potential and whether this is consistent across different sample types. HTGC chromatograms of each sample fraction will provide a visual image of equivalent fractions obtained from different samples. Integration of chromatographic data, even without appropriate standards, will provide an assessment of the relative proportion of constituents (e.g., resolved peaks, UCMs, or carbon number group integrals) in common chemical classes and can be related back to the weight percentage data.
- B. Wax analysis: Data from wax analysis will be presented as gravimetric weight obtained from topping procedure and weight percentage individual *n*-alkanes. If required, the data can be compiled as weight percentage group intervals based on carbon number to account for compounds that behave as 'waxes' but are not *n*-alkanes.

## Relevant Papers

Nelson, K.N., Scarlett, A.G., Monique Gagnon, M., Holman, A.I., Reddy, C.M., **Sutton, P.A.** and Grice, K. (2022) Characterizations and comparison of low sulfur fuel oils compliant with 2020 global sulfur cap regulations for international shipping. *Marine Pollution Bulletin* **180**, 113791 [doi: <https://doi.org/10.1016/j.marpolbul.2022.113791>].

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Robson, W.J. (2018) The separation of the polar constituents of petroleum. PhD Thesis, University of Plymouth, UK. (**Co-Supervisor**).

Robson, W.J., **Sutton, P.A.**, McCormack, P., Chilcott, N.P. and Rowland, S.J. (2017) Class type separation of the polar and apolar components of petroleum. *Analytical Chemistry* **89**, 2919-2927. [doi: [10.1021/acs.analchem.6b04202](https://doi.org/10.1021/acs.analchem.6b04202)].

**Sutton, P.A.** and Rowland, S.J. (2014) Determination of the content of C<sub>80</sub> tetraacids in petroleum. *Energy & Fuels* **28**, 5657-5669. [doi: [10.1021/ef5012337](https://doi.org/10.1021/ef5012337)].

**Sutton, P.A.** and Rowland, S.J. (2012) High temperature gas chromatography-time-of-flight-mass spectrometry (HTGC-ToF-MS) for high-boiling compounds. *Journal of Chromatography A*. **1243**, 69-80. [doi: [10.1016/j.chroma.2012.04.044](https://doi.org/10.1016/j.chroma.2012.04.044)].

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**Sutton, P.A.**, Smith, B.E., Waters, D. and Rowland, S.J. (2010) Identification of a novel ester obtained during isolation of C<sub>80</sub> ("ARN") tetraprotic acids from an oilfield pipeline deposit. *Energy Fuels* **24**, 5579-5585. [doi: [10.1021/ef1008743](https://doi.org/10.1021/ef1008743)].

Booth, A., **Sutton, P.A.**, Lewis, C.A., Lewis, A.C., Scarlett, A., Wing Chau, Widdows, J. and Rowland, S.J. (2007) Unresolved complex mixtures of aromatic hydrocarbons: Thousands of overlooked persistent, bioaccumulative and toxic contaminants in mussels. *Environmental Science & Technology* **41**, 457-464. [doi: [10.1021/es0615829](https://doi.org/10.1021/es0615829)].

Smith, B.E., **Sutton, P.A.**, Lewis, C.A., Dunsmore, B., Fowler, G., Krane, J., Lutnaes, B.F., Brandal, Ø., Sjöblom, J. and Rowland, S.J. (2007) Analysis of 'ARN' naphthenic acids by high temperature gas chromatography and high performance liquid chromatography. *Journal of Separation Science* **30**, 375-380. [doi: [10.1002/jssc.200600266](https://doi.org/10.1002/jssc.200600266)].

**Sutton, P.A.**, Lewis, C.A. and Rowland S.J. (2005) Isolation of individual hydrocarbons from the unresolved complex hydrocarbon mixture of a biodegraded crude oil using preparative capillary gas chromatography. *Organic Geochemistry* **36**, 963-970. [doi: [10.1016/j.orggeochem.2004.11.007](https://doi.org/10.1016/j.orggeochem.2004.11.007)].