

**Biodegradation at the Seafloor: Ultrahigh Resolution FT-ICR Mass Spectral
Characterization of Natural Petroleum Seeps**

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ABSTRACT 300239:

We report the first resolution, identification, and characterization of nickel and vanadyl porphyrins simultaneously in weathered oil samples derived from natural seeps in Southern California. Atmospheric pressure photoionization (APPI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) provides ultrahigh resolving power ($m/\Delta m_{50\%} > 1,000,000$ at m/z 500) and sub-ppm mass error (<50 ppb) required to identify nickel porphyrin isotopes for unambiguous elemental composition assignment. We also report the first simultaneous identification and categorization of both vanadyl and nickel porphyrins in the same sample, without prior sample fractionation. More than 85,000 mass spectral peaks are resolved and identified in a single mass spectrum, and represent the most extensive molecular deconvolution of an organic mixture characterized to date.

INTRODUCTION:

Crude oil released through natural petroleum seeps accounts for nearly half of the oil that enters coastal and oceanic environments,¹ with estimated global input of ~ 600,000 metric tons released annually through natural seepage.² Natural oil seepage releases more oil into the marine environment than human activities (i.e., petroleum exploration, production, and transportation), and catastrophic failures in man-made storage/transport vessels (tankers, pipelines) combined.² Natural petroleum seeps along the coast of Southern California have been the subject of numerous studies on marine hydrocarbon seeps.^{1, 3-12}

Petroporphyrins constitute a unique class of compounds that derive from chlorophyll and heme structures, and were the first molecules that linked crude oil to its biological origin.^{13, 14} The porphyrin core structure is a planar, cyclic molecule that contains four pyrrolic nitrogen linked with methine bridges, and substitution of the eight β -hydrogens results in different structures that exist as a homologous series in petroleum.¹⁵ During crude oil maturation, the phytyl chain from chlorophyll is lost, and magnesium is replaced by nickel or vanadyl ion. Nickel and vanadyl porphyrins are the most abundant metal compounds in petroleum, and are used in petroleum classification¹⁶ and as maturity indicators.¹⁷ The concentration of vanadium and nickel vary with oil composition, geographic origin, and marine depositional environment.¹⁸ Most porphyrins originate from different types of chlorophyll, and provide highly specific paleoenvironmental information.¹⁹⁻²¹ For example, Gibbison *et al.*,²² identified $>C_{32}$ carbon skeletons of porphyrins derived from sedimentary rock that derived from bacteriochlorophyll d of *Chlorobiaceae*, an obligate anaerobic bacteria, and indicate anoxic conditions during source rock deposition.²⁰

Nickel and vanadyl petroporphyrin content increases with viscosity, and heavy crude oil and distillates can contain >1200 ppm vanadium.²³ The extraordinary compositional complexity associated with high boiling crude oils and bitumens challenges nearly all analytical techniques.²⁴⁻²⁶ Only Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry can address the compositional complexity associated with heavy oils at the molecular level.²⁷⁻³¹ Decades of scientific literature have explored vanadium and nickel porphyrin chemistry,³²⁻³⁴ separation and isolation techniques,³⁵⁻³⁹ and mass analysis.⁴⁰⁻⁴⁷ The main porphyrin structures identified in petroleum correspond to etioporphyrin (Etio) and deoxophylloerythroetioporphyrin (DPEP) structures complexed in nickel and vanadyl forms,^{13, 14} and recent reports have identified sulfur- and oxygen-containing porphyrins.^{16, 18, 38, 48}

Nickel (II) and oxo-vanadium(IV) complexes of deoxophylloerythroetioporphyrin are the most abundant petroporphyrins found in petroleum and bitumen.^{42, 49} Petroporphyrins were the first compound class recognized as biomarkers because the carbon skeleton is preserved through incorporation into sediments during diagenesis.⁴⁰ Petroporphyrins are one (and possibly the only) class of petrocompounds that are *not* continuous in carbon number, aromaticity, and heteroatom content as a function of boiling point.^{27-30, 50-54} Direct characterization of vanadyl and nickel porphyrins in petroleum requires advanced analytical techniques because their 25 to 60 carbon atoms^{15, 16} that challenge resolution with GC-based techniques.⁵⁵ The structural

characterization of trace metals in crude oil is critical for petroleum processing, because nickel and vanadium poison catalysts used in catalytic upgrading of heavy oil, but few studies have characterized metalloporphyrins from the environmental perspective.⁴⁹ Cantu *et al.* reported low partitioning of nickel and vanadyl DPEP porphyrins from Boscan crude oil into the aqueous phase that is enhanced with surfactants (like humic substances). To the best of our knowledge, no study has characterized the structural diversity petroporphyrins derived from natural petroleum seeps.

As the global oil supply shifts towards heavy crudes, refinery feeds will contain an increased amount of trace metals. Development of efficient demetallation strategies requires detailed, molecular-level structural characterization.³² Heavily weathered, highly degraded natural petroleum seeps are arguably the most compositionally diverse, naturally occurring hydrocarbon matrix. Identification of individual compounds classes, such as nickel and vanadyl porphyrins, challenges nearly all analytical techniques. Only FT-ICR MS can address the compositional diversity of heavily weathered seep samples, and resolve and identify the two main types of petroporphyrins simultaneously without separation from the complex hydrocarbon.

We previously reported the first direct identification of vanadyl porphyrins at the molecular level from South American heavy crude oil and Athabasca bitumen asphaltenes.⁴⁸ Qian *et al.* observed vanadyl porphyrins, sulfur-containing vanadyl porphyrins⁴⁵ and nickel porphyrins⁴⁴ by APPI FT-ICR MS, but required extensive solubility and cyclograph fractionation prior to detection. Zhao *et al.* developed a method to sequentially fractionate Venezuelan heavy crude and reported three new series of vanadyl porphyrins by positive-ion electrospray ionization FT-ICR MS.³⁸ However, vanadyl and nickel porphyrins have not previously been identified simultaneously. Here, we combine atmospheric pressure photoionization (APPI) FT-ICR MS to directly identify and catalogue both vanadyl and nickel porphyrin structural diversity in heavily weathered natural seeps from Santa Barbara basin in the same sample.

METHODS:

Sample Description

Asphalt samples of *Il Duomo* (#4332-1), discovered in 2007 off the coast of southern California in the Santa Barbara basin, are detailed elsewhere.⁵⁶

Sample Preparation

The *Il Duomo* sample was finely ground in a mortar and pestle. Carptineria seep samples were collected as chunks of oil and sand, and extracted with 90:10 DCM:methanol. Both samples were dissolved in toluene to make a stock solution (500 µg/mL). Prior to APPI FT-ICR MS analysis, samples were diluted to a final concentration of 125 µg/mL with no additional modification.

Ionization

An atmospheric pressure photoionization (APPI) source (Thermo-Fisher Scientific, San Jose, CA) was coupled to the first stage of a custom-built FT-ICR mass spectrometer (see below) through a custom-built interface.⁵⁷ The tube lens was set to 50 V (to minimize ion fragmentation)

and heated metal capillary current was 4.5 A. A Hamilton gas-tight syringe (5.0 mL) and syringe pump were used to deliver the sample (50 $\mu\text{L}/\text{min}$) to the heated vaporizer region (350 $^{\circ}\text{C}$) of the APPI source, where N_2 sheath gas (50 psi) facilitates nebulization. The auxiliary port remained plugged. After nebulization, gas-phase neutral analyte molecules exit the heated vaporizer region as a confined jet. A krypton vacuum ultraviolet gas discharge lamp (Syagen Technology, Inc., Tustin, CA) produces 10-10.2 eV photons (120 nm). Toluene increases the ionization efficiency for nonpolar aromatic compounds through dopant-assisted APPI^{58, 59} through charge exchange,^{60, 61} and proton transfer⁶² reactions between ionized toluene molecules and neutral analyte molecules. Protonated ions exhibit half-integer double bond equivalents values ($\text{DBE} = c - h/2 + n/2 + 1$, calculated from ion elemental composition $\text{C}_c\text{H}_h\text{N}_n\text{O}_o\text{S}_s$),⁶³ and may thus be distinguished from radical cations with integer DBE values.^{48, 57}

Mass analysis

Il Duomo and Carpinteria samples were analyzed with a custom-built FT-ICR mass spectrometer⁶⁴ equipped with a 22 cm horizontal room temperature bore 9.4 T superconducting solenoid magnet (Oxford Instruments, Abingdon, U.K.), and a modular ICR data station (Predator)⁶⁵ facilitated instrument control, data acquisition, and data analysis. Positive ions generated at atmospheric pressure enter the skimmer region (~ 2 Torr) through a heated metal capillary, pass through the first radiofrequency (rf)-only octopole, pass through an rf-only quadrupole, and are externally accumulated⁶⁶ (25-50 ms) in a second octopole equipped with tilted wire extraction electrodes for improved ion extraction and transmission.⁶⁷ Helium gas introduced during accumulation collisionally-cools ions prior to transfer through rf-only quadrupoles (total length 127 cm) (into a 7-segment open cylindrical cell with capacitively-coupled excitation electrodes based on the Tolmachev configuration.^{68, 69} 150-200 individual transients of 6.8 s duration were signal averaged. The data was collected at the maximum memory depth of the data station hardware 16 million samples, apodized with a single sided Hanning apodization, zero filled to 16 mega sample (16777216 samples or 2^{24}). This small zero fill is required to facilitate the fast Fourier transform algorithm. An additional zero fill brings the preFT data packet to 32 mega sample, which in turn is processed via absorption FT analysis.^{70, 71} For all mass spectra, the achieved spectral resolving power approached the theoretical limit⁷² over the entire mass range, e.g., average resolving power, $m/\Delta m_{50\%}$, in which $\Delta m_{50\%}$ is mass spectral peak full width at half-maximum peak height was $\sim 1,000,000 - 1,500,000$ at m/z 500.

Mass Calibration and Data Analysis

ICR frequencies were converted to ion masses based on the quadrupolar trapping potential approximation.^{73, 74} Each m/z spectrum was internally calibrated with a “walking” calibration equation with respect to an abundant homologous alkylation series whose members differ in mass by integer multiples of 14.01565 Da (mass of a CH_2 unit) confirmed by isotopic fine structure.⁷⁵ Experimentally measured masses were converted from the International Union of Pure and Applied Chemistry (IUPAC) mass scale to the Kendrick mass scale to identify homologous series for each heteroatom class (i.e., species with the same $\text{C}_c\text{H}_h\text{N}_n\text{O}_o\text{S}_s$ content, differing only by the degree of alkylation). Peak assignments were performed by Kendrick mass defect analysis as previously described.⁷⁶

Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

All samples were completely digested in a high pressure asher at 300 °C for 5 h at 100 bar in concentrated nitric acid. The resulting digestates **were diluted** and analyzed by ICP-MS with a ThermoScientific, Inc. Element 2 instrument operated in medium resolution mode and quantitated based on standard addition. **Table 1** reports vanadium and nickel concentrations for *Il Duomo* asphalt volcano and Carpinteria seep samples.

Table 1. Elemental composition of *Il Duomo* asphalt volcano and Carpinteria terrestrial seep samples.

Sample	V (ppm)	Ni(ppm)
Carpinteria 1.2	554 (+/-5)	950 (+/-9)
Il Duomo 4332-1	514 (+/-2)	602 (+/-6)

Trace metals (<1000 mg/kg) in petroleum include iron, zinc, nickel, vanadium, cobalt, chromium, and copper, and reflect metals incorporated into porphyrin complexes through migration or in the reservoir through source rock and biomass sedimentation.^{35, 41, 77} Trace metal concentration, distribution and content in crude oils report information on origin, migration, deposition environment and oil maturity.⁷⁸ In particular, nickel and vanadium ratios classify oils of different geographic origin, especially when source rock data is limited or not available.¹⁶ Crude oil from terrestrial organic matter has V/Ni ratios = 1-10, and V/Ni ratios < 0.5 correspond to marine organic matter-derived crude oils.¹⁶ Both *Il Duomo* and Carpinteria samples report V/Ni ratios 0.58 and 0.85, which correspond to crude oil formed from marine organic matter.

RESULTS AND DISCUSSION:

Figure 1 shows a broadband positive-ion APPI FT-ICR mass spectrum for an *Il Duomo* asphalt volcano sample. The achieved resolving power $m/\Delta m_{50\%} = 1,400,000$ at m/z 515 enables resolution of 85,920 mass spectral peaks, each with magnitude greater than 6σ of baseline rms noise (m/z 200-1100) with a mass distribution centered at m/z 400. The mass scale-expanded segment at m/z 515 shows ~171 peaks. The theoretical resolving power required to separate two equally abundant species that differ in mass by ~548 μ Da at 9.4 tesla is 890,000. The presently achieved resolving power ($m/\Delta m_{50\%} = 1,400,000$ at m/z 515) enables separation of species that differ in mass by $C_1N_1^{13}C_1$ versus $H_5^{34}S_1$, both of nominal mass 39 Da, and differing in mass by 530 μ Da—i.e., less than the mass of an electron (548 μ Da).⁷⁹⁻⁸¹ To the best of our knowledge, this mass spectrum represents the most peaks resolved and identified in a single spectrum of any kind, surpassing our previous record,²⁷ and represents the highest broadband resolving power for any petroleum mass spectrum, and emphasizes the need for ultrahigh resolving power achievable only by FT-ICR MS sufficient to separate isobaric overlaps prevalent in complex seep samples.

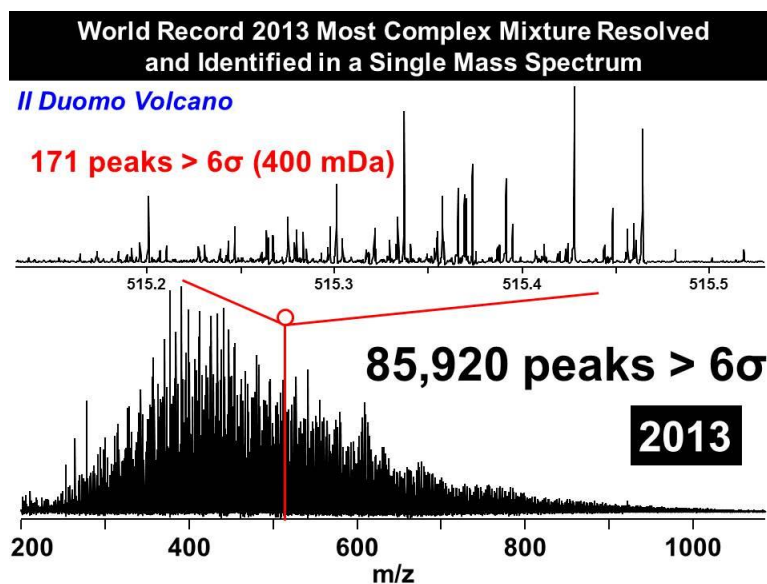


Figure 1. Positive-ion APPI FT-ICR MS of a sample from the *Il Duomo* asphalt volcano. Top: Time-domain ICR signal. Bottom: Broadband FT-ICR mass spectrum, containing more than 85,000 resolved peaks, each of magnitude greater than 6 σ of baseline noise. Middle: 400 mDa mass scale-expanded segment, showing resolution of two elemental compositions differing in mass by ~ 0.5 mDa (i.e., the mass of one electron).

Nickel Petroporphyrin Structural Diversity

Relative abundances of two *Il Duomo* nickel porphyrins differing in double bond equivalents, and therefore, different in structure. The DBE value of 18 corresponds to a DPEP ($C_nH_{2n-30}N_4$) structure that is the most abundant *Il Duomo* nickel porphyrin, and the DBE = 17 species corresponds to the etioporphyrin structure ($C_nH_{2n-30}N_4$). Here, nickel porphyrins are resolved from isobaric overlaps and confirmed by isotopic fine structure. Mass resolving power in excess of 1,000,000 enables elemental composition assignment of monoisotopic (^{58}Ni) nickel porphyrins directly from a broadband FT-ICR mass spectrum, and validates those assignments from isotopic fine structure of their ^{60}Ni isotopologues, without the need for prior fractionation.

Relative Abundances of Nickel and Vanadyl Petroporphyrins

Although nickel concentration is higher in *Il Duomo* (Table 1), vanadyl porphyrins are detected in higher relative abundance than nickel counterparts. Based on model compounds, Qian *et al.* reported three times higher signal magnitude for vanadyl porphyrins relative to nickel porphyrins at 600 ppm by APPI FT-ICR MS, attributed to lower ionization efficiency for nickel porphyrins. However, their model compounds, octaethyl-VO porphyrin and octaethyl-Ni porphyrin, correspond to the etio porphyrin (DBE = 17) structure. They reported that low maturity and severely biodegraded crude oils typically contain “total vanadium content between 400- >1000ppm, while total nickel content rarely exceeds 100 ppm”, and conclude that “APPI FT-ICR MS sensitivity is not sufficient to direct Ni porphyrins directly”, due to dynamic range limitations ($<10^4$) of FT-ICR MS that limits direct identification. Here, we provide the first direct

identification of both nickel and vanadyl porphyrins, and resolve isobaric overlaps to confirm elemental compositions with isotopic fine structure simultaneously in a broadband APPI FT-ICR mass spectrum from the *Il Duomo* asphalt volcano that contains nearly equal amounts of total nickel and vanadium ($\text{Ni/V} = 1.17$).

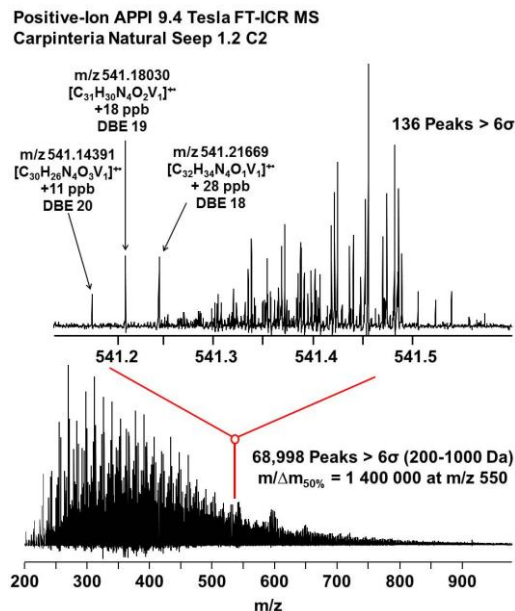


Figure 2. Bottom: Broadband positive-ion APPI FT-ICR mass spectrum of fresh oil collected from a Carpinteria beach. Top: Mass scale-expanded segment at 541 Da nominal mass, enabling identification of oxygen-containing vanadyl porphyrins.

Direct Identification of New Vanadyl Porphyrin Structures

Vanadyl porphyrins have previously been reported in raw asphaltenes and heavy crude oil by FT-ICR MS without prior separation or enrichment.⁴⁸ **Figure 2** shows a broadband positive-ion APPI FT-ICR mass spectrum of fresh oil collected from Carpinteria beach. The achieved resolving power of 1,400,000 at m/z 515 results in 68,988 mass spectral peaks, each with magnitude greater than 6σ of baseline rms noise (m/z 200-1000) and mass distribution centered at m/z 350-400. The mass scale-expanded segment at m/z 541 shows the monoisotopic peak for a DPEP vanadyl porphyrin (DBE = 18) at m/z 541.21669 identified to within ~ 30 ppb.⁴⁸ (Two additional monoisotopic peaks correspond to DPEP vanadyl porphyrins with one $[\text{C}_{31}\text{H}_{30}\text{N}_4\text{O}_2\text{V}_1]^{+}$ or two $[\text{C}_{30}\text{H}_{26}\text{N}_4\text{O}_3\text{V}_1]^{+}$ oxygen atoms and are identified within 20 ppb. Zhao *et al.* first identified three vanadyl porphyrin series ($\text{C}_n\text{H}_m\text{N}_4\text{VO}_2$, $\text{C}_n\text{H}_m\text{N}_4\text{VO}_3$ and $\text{C}_n\text{H}_m\text{N}_4\text{VO}_4$) by FT-ICR MS, but required extensive solvent extraction and chromatographic fractionation to separate vanadyl porphyrins from Venezuelan Orinoco heavy crude.³⁸ Here, two new series of vanadyl porphyrins are identified at the molecular level in a natural petroleum seep sample derived from Carpinteria beach, without prior separation.

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