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1 **Comprehensive two-dimensional liquid chromatography with**  
2 **inductively coupled plasma mass spectrometry detection for the**  
3 **characterization of sulfur, vanadium and nickel compounds in petroleum**  
4 **products**

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12

13 **Abstract**

14 The petroleum industry is increasingly concerned with the conversion of vacuum residues as  
15 a consequence of decreased conventional crude oil availability. The compositional analysis of  
16 heavy oil products has become a key step in conversion processes, but the complexity of  
17 these oil matrices tends to increase with their boiling point. In this study, comprehensive  
18 two-dimensional liquid chromatography (LCxLC) coupled to inductively coupled mass  
19 spectrometry (ICP-MS/MS) is considered with a view to meet new requirements and to bring  
20 additional information regarding the species present in these matrices. In search for a high  
21 degree of orthogonality, two separation techniques involving two different retention  
22 mechanisms were evaluated: Size Exclusion Chromatography (SEC) and Reverse Phase Liquid  
23 Chromatography (RPLC). In SEC, the analytes are separated according to their molecular  
24 weight while according to their hydrophobicity in RPLC. The separation power of both  
25 individual separation techniques was first evaluated. Off-line and on-line LCxLC were  
26 compared on the basis of an optimization approach. It is shown that off-line SECxRPLC can  
27 provide, for the same analysis time of 150 min, a higher peak capacity (2600 vs 1700) while a  
28 similar dilution factor (close to 30) but also requires far fewer fractions to be analyzed (12 vs

29 400). Asphaltenes which constitute the heaviest fraction of crude oils (obtained from  
30 petroleum industry) were analyzed by the developed off-line SECxRPLC method. The  
31 resulting 2D-contour plots show that co-elutions could be removed leading, for the first  
32 time, to new information on high molecular weight species containing sulfur and vanadium.

### 33 **Keywords:**

34 SECxRPLC; Peak capacity; Heavy oils; Organic matrices; Speciation; ICP-MS/MS

35

## 36 **1. Introduction**

37 As the production of conventional middle and light oils has been declining over the last  
38 decades, heavy crude oils are increasingly used in the petroleum industry to replace them  
39 [1]. Crude oil is a complex mixture containing heteroatoms such as sulfur, and heavy metals  
40 like nickel and vanadium [2]. Vanadium and nickel are known to be present in petroleum up  
41 to few hundred parts per million [3,4]. To improve demetallation and hydrodesulfurization  
42 processes, a better understanding of metal speciation is necessary, which has led to a  
43 growing interest in hyphenated techniques, which can combine the high separation power of  
44 ultra-high performance liquid chromatography (UHPLC) to a specific detection such as  
45 inductively coupled plasma mass spectrometer (ICP-MS/MS). Up to now, the structural  
46 identification of the chemical species present in the matrix is far from being comprehensive.  
47 Porphyrins, chelated with nickel and vanadyl, are widely described in the literature [5–9].  
48 However, other compounds which represent up to 80 % of the metal complex, are much  
49 more abundant than metalloporphyrins while poorly described yet [3,6], and could be found  
50 as porphyrins trapped in nanoaggregates [10,11]. Recently, several reviews have dealt with  
51 metal speciation in crude oil and heavy petroleum fraction [2,3,12]. One of the main  
52 reported analytical approaches is size exclusion chromatography coupled to ICP-HRMS (SEC-  
53 ICP-HRMS) [10,13–17]. The compounds being separated according to their hydrodynamic  
54 volumes, the catalyst pore size can be adapted according to the size of metal complexes,  
55 which can be useful for hydrodemetallation and hydrodesulfurization processes [3]. The  
56 resulting elution profiles usually consist in up to three large unresolved peaks [18,19].  
57 Reverse Phase Liquid Chromatography (RPLC) separation was also reported for the analysis

58 of metalloporphyrins [20], and asphaltene fractions [21]. From all reported studies, it is clear  
59 that one-dimensional chromatography fails to provide enough separation power for a  
60 complete characterization of such very complex matrices. A few approaches were developed  
61 using two-dimensional liquid chromatography (2D-LC) for the analysis of vanadium and  
62 nickel. They combined size exclusion chromatography and Normal Phase Liquid  
63 Chromatography (NPLC) to get relevant information on both molecular weight and chemical  
64 specie polarity. Three [22] or four fractions [23] were collected from the SEC dimension and  
65 further sent to the second NPLC dimension. Although this analytical approach did not allow  
66 the complete speciation of crude oils, it could point out three metal-groups of different  
67 polarities.

68 In the present study, we explored the feasibility and the potential of comprehensive two-  
69 dimensional liquid chromatography (SEC combined with RPLC) coupled to ICP-MS/MS for a  
70 better characterization of such complex matrices. With organic matrices, the presence of  
71 organic solvents requires the addition of oxygen into the plasma to avoid carbon deposit  
72 onto the cone surface and plasma instabilities. In addition to this, a more critical issue with  
73 RP gradients in the second dimension stems from the continuous change in solvent  
74 composition, resulting in signal fluctuations during the run. Although different approaches  
75 were proposed, such issue is still partly solved [24–30]. For instance, some authors  
76 suggested the use of a post column counter gradient prior to ICP-MS detection [29], or  
77 isotope dilution [30]. A 2D-configuration with RPLC (gradient conditions) in the first  
78 dimension and SEC (isocratic conditions) in the second one is expected to be more attractive  
79 with respect to both separation power and peak intensities considering that, under isocratic  
80 conditions, (i) plasma instabilities are less significant and (ii) optimized plasma parameters  
81 are easier to find. A theoretical study was therefore carried out to select the best  
82 comprehensive 2D-configuration (SECxRPLC or RPLCxSEC). We made use of a calculation  
83 approach previously developed for optimizing physical parameters in LCxLC with a view to  
84 maximizing the peak capacity while minimizing the dilution within a given analysis time  
85 [31,32]. This approach also allowed us to choose between on-line and off-line mode.  
86 Whereas the off-line mode is more time-consuming, less suitable for automation, and more  
87 prone to sample loss and carry over, it should offer some advantages for our purpose: (i) a  
88 simple conventional HPLC systems can be used, (ii) after collection, the injection of the

89 fractions into the second dimension can be delayed making a fraction treatment possible,  
90 (iii) the same instrument can be used in both dimensions and above all (iv) the analysis time  
91 in the second dimension is not limited [34].

92 The first part of this work was devoted to the selection of appropriate conditions for the first  
93 and the second dimensions, both in off-line and on-line mode. As a result of this  
94 optimization procedure, off-line SECxRPLC-ICP-MS/MS was applied to the analysis of the  
95 sub-fractions of heavy crude oils. The obtained results could provide new information on  
96 high molecular weight species containing sulfur and vanadium.

## 97 **2. Experimental section**

### 98 **2.1. Chemicals and reagent**

99 Tetrahydrofuran (LC-MS grade), acetonitrile (LC-MS grade), 2,3,7,8,12,13,17,18-Octaethyl-  
100 21H,23H-porphine vanadium(IV) oxide standard were purchased from Sigma Aldrich  
101 (Steinheim, Germany). Ethyl acetate was purchased from Carlo Erba (Val-de-Reuil, France).  
102 1-Methyl-2-pyrrolidinone were purchased from Fisher Scientific (Illkirch, France). Water was  
103 obtained from Elga water purification system (Veolia water STI, Le Pless Robinson, France).  
104 SPEX CertiPrep (Metuchen, NJ, USA) Co, Ce, Y, Tl, Li monoelemental standards in 2 % HNO<sub>3</sub>  
105 (1000 µg/L) were used daily for the ICP-MS/MS system calibration. A multi-element solution  
106 containing Co, Ce, Y, Tl, Li at 10 ppb in acetonitrile was used to monitor the instrument. 8  
107 polystyrene standards were used in 2 different solutions. Solution A contains PS of 28000,  
108 9130, 682 and 266 Da, solution B contains PS of 44000, 15700, 3470, and 1250 Da in THF.  
109 Polystyrene standards were supplied by Fluka Analytical, Sigma Aldrich (Steinheim,  
110 Germany).

### 111 **2.2. Sample preparation**

112 Analyzed samples came from Vacuum Residues (VRs) obtained after atmospheric distillation  
113 and vacuum distillation in the refining process of crude oil. To simplify the matrix, VRs have  
114 been fractionated according to a two-step SARA method [3], as shown in Fig.1. Asphaltenes  
115 were first precipitated with a non-polar solvent (n-heptane). The fractionation of the  
116 maltenes was then performed by column chromatography resulting in SARA fractions  
117 (Saturates, Aromatics, Resins, and Asphaltenes). Those were supplied by IFPEN. SARA

118 fractions were then diluted 60-times v/v in THF before being analyzed. An atmospheric  
119 residue (AR Venezuela), supplied by IFPEN and diluted 60 times, was also used for the  
120 evaluation of the performance of SEC-ICP-MS.

## 121 **2.3. Instrumentation**

### 122 **2.3.1. LC system**

123 The instrument was an Infinity 1290 LC from Agilent Technologies (Waldbronn, Germany). It  
124 includes a high-pressure binary solvent manager with a maximum flow-rate of 5 mL/min, an  
125 auto-sampler with a flow-through needle device equipped with a 20  $\mu$ L sample loop, a  
126 column oven with a maximum temperature of 100 °C. The measured dwell volume was  
127 300  $\mu$ L. The instrument control was performed by Mass Hunter. A UV detector equipped  
128 with 2  $\mu$ L flow-cell was used for experiment described section 3.1. Detector wavelength was  
129 set at 407 nm. The same LC instrument was used in the two dimensions. The outlet of the  
130 column was connected to a T-union P-727 (from Upchurch, Cluzeau, Sainte-Foy-La-Grande,  
131 France) with zero dead volume. Only stainless steel tubing was used for THF compatibility.  
132 Tube #1 (50 cm x 125  $\mu$ m) was located between the column and the T-union, Tube #2 (20 cm  
133 x 125  $\mu$ m) between the T-union and the Sample Introduction System (SIS) prior to the ICP-  
134 MS/MS, Tube #3 (25 cm x 125  $\mu$ m) between the T-union and the waste. The split ratio was  
135 set to 1:1 to ensure proper conditions (400  $\mu$ L/min) for the nebulizer. SEC column calibration  
136 was performed with a diode array detector equipped with 0.6  $\mu$ L flow-cell, from Agilent  
137 Technologies (Waldbronn, Germany).

### 138 **2.3.2. ICP-MS/MS**

139 The experiments were carried out using an Agilent 8800 ICP-MS/MS system from Agilent  
140 Technologies (Waldbronn, Germany) with a PFA-LC nebulizer from Elemental Scientific  
141 (Omaha, Nebraska USA), an IsoMist device from Courtage Analysis Service (Mont Saint  
142 Aignan, France), equipped with a Twinnabar spray chamber as Sample Introduction System  
143 (SIS), selected according to previous studies [35,36]. The spray chamber was thermostated at  
144 -2 °C by Peltier effect. The system was equipped with a quartz torch with a 1 mm i.d. injector  
145 (Agilent Technologies). The following ICP-MS/MS parameters were selected: plasma power  
146 of 1500 W, auxiliary gas flow-rate of 0.9 L/min, plasma gas flow-rate of 15 L/min, sampling  
147 depth of 7 mm, carrier gas of 0.45 L/min. Viton<sup>®</sup> tubing (SCP Science, Villebon-sur-Yvette,

148 France) were used for correct use of the peristaltic pump under organic solvent conditions.  
149 In order to ensure plasma stability and to avoid carbon deposit on the cone surface (sampler  
150 and skimmer), 0.5 L/min of a mixture of Oxygen and Argon (20 % O<sub>2</sub>, 80% Ar) was sent  
151 through HMI tubing into ICP-MS/MS. Both sampling and skimmer cones were made of  
152 platinum, due to its inertness, instead of usual nickel. The amount of oxygen, depending on  
153 the mobile phase (type of organic solvent and flow-rate) was optimized by visually  
154 monitoring C<sub>2</sub> emission band. The analyzer unit of the instrument consisted of two  
155 quadrupole mass analyzers and an octopole collision-reaction cell located between them.  
156 Vanadium, sulfur, and nickel were detected in MS/MS mode with O<sub>2</sub> as reaction gas in the  
157 cell. Vanadium 51 was shifted to mass 67 (<sup>67</sup>VO<sup>+</sup>), Sulfur 32 to mass 48 (<sup>48</sup>SO<sup>+</sup>). Nickel was  
158 analyzed at m/z = 58 (<sup>58</sup>Ni<sup>+</sup>).

159 To maximize the instrument sensitivity, the flow-rate of O<sub>2</sub> reaction gas was optimized by  
160 monitoring <sup>67</sup>VO<sup>+</sup> intensity of a porphyrin sample (1 ppm in ACN) while changing the mobile  
161 phase flow-rate within 0.1 to 0.8 mL/min (see supplementary information S1). The highest  
162 sensitivity was achieved at 0.3 mL/min O<sub>2</sub>.

163 The same approach was applied to the nickel and sulfur elements. O<sub>2</sub> reaction gas flow-rate  
164 was optimized by monitoring both <sup>48</sup>SO<sup>+</sup> and <sup>58</sup>Ni<sup>+</sup> intensities (100 ppb of mono-elemental  
165 standards of nickel and sulfur diluted in THF) (see supplementary information S1). The  
166 highest sensitivity was achieved at 0.2 and 0.25 mL/min O<sub>2</sub> respectively. The MS/MS mode  
167 was essential for sulfur analysis to avoid isobaric interference such as O<sub>2</sub><sup>+</sup>. As a result, the  
168 analysis of the three elements (sulfur, vanadium, and nickel) could be performed within one  
169 single run at 0.3 mL/min O<sub>2</sub>.

### 170 **2.3.3. SEC-ICP-MS/MS setup**

171 Two Waters APC columns were used: an ACQUITY APC XT 45 (150 x 4.6 mm, 1.7 μm) with  
172 effective molecular weight ranging from 200 to 5000 g/mol and an ACQUITY APC XT 125  
173 (150 x 4.6 mm, 2.5 μm) with effective molecular weight ranging from 1000 to 30000 g/mol.  
174 For both columns, the injection volume was 15 μL. The column temperature was set to  
175 30 °C. THF was used as mobile phase. The mobile phase flow-rate was 0.8 mL/min. A flow-  
176 splitter (1:1) was used prior to the ICP-MS/MS detector.

### 177 **2.3.4. RPLC-ICP-MS/MS**

178 Several stationary phases were evaluated in RPLC. Those are listed in Table S1. The column  
 179 temperature was set at 30 °C. Acquity BEH C<sub>18</sub>, Vydac C<sub>18</sub>, Hypersil Betabasic were operated  
 180 at a flow-rate of 0.8 mL/min. Zorbax-SB-CN was operated at a flow-rate of 1 mL/min. 80/20  
 181 ACN/H<sub>2</sub>O (v/v) was used as mobile phase (A) and THF was used as mobile phase (B). A linear  
 182 gradient was applied from 1% B to 99% B, keeping a normalized gradient slope of 5 %  
 183 (gradient slope x column dead time). The injection volumes are given in Table S1. Flow-  
 184 splitting (1:1) was used prior to the ICP-MS/MS detector.

### 185 **2.3.5. SECxRPLC-ICP-MS/MS setup**

186 In off-line SECxRPLC, an ACQUITY APC XT 125 (150 x 4.6 mm, 2.5 μm) column and an  
 187 ACQUITY BEH C<sub>18</sub> (50 x 2.1 mm, 1.7 μm) column were used in the first (<sup>1</sup>D) and second (<sup>2</sup>D)  
 188 dimensions respectively. The injection volumes were 15 μL and 5 μL respectively. The  
 189 column temperature was set at 30 °C in both dimensions. <sup>1</sup>D mobile phase was THF. <sup>2</sup>D  
 190 mobile phase was ACN/H<sub>2</sub>O, 80/20, (v/v) (A), and THF (B) with the following gradient  
 191 program: 0-10.2 min, 1 % B to 99 % B; 10.2-15.2 min, 99 % B; 15.2-15.3 min, 99 % to 1 % B;  
 192 15.3-15.8 min, 1 % B, leading to a normalized gradient slope of 1.2 %. In both dimensions the  
 193 flow-rate was 800 μL/min with a flow-splitter of 1:1 prior to the ICP-MS/MS detection.  
 194 Fractionation was manually performed every 10 s since no fraction collector was compatible  
 195 with THF at the time of the present study. The filled vials were directly placed into the auto-  
 196 sampler to be further analyzed in the second dimension. No fraction treatment was  
 197 performed between both dimensions.

## 198 **2.4. Calculations**

199 The peak capacity,  $n$ , was first introduced by Giddings to provide a valuable tool capable of  
 200 assessing the separation power. It was defined as the number of peaks that can be ideally  
 201 placed between the first peak and the last peak of interest with a resolution of 1.0 between  
 202 all peaks [37]. In RPLC and in gradient elution, the peak capacity,  $n_{RPLC}$ , can be calculated  
 203 according to [38]:

$$204 \quad n_{RPLC} = 1 + \frac{\sqrt{N_{measured}}}{4} \cdot \frac{1}{2.3b+1} \cdot \ln \left( \frac{2.3b+1}{2.3b} \cdot e^{2.3S\Delta C_e} - \frac{1}{2.3b} \right) \quad (1)$$

205 Where  $S$  is the average slope of the relationship between the logarithm of the retention  
 206 factor and the solvent composition. It can be estimated from the molecular mass, according



207 to an empirical relationship,  $\Delta C_e$  is the range of compositions at elution covered by the  
 208 sample,  $N_{measured}$ , the measured plate number which may differ from the column plate  
 209 number due to extra-column dispersion [32] and  $b$  is the Linear Solvent Strength (LSS)  
 210 gradient steepness [39], defined as:

$$211 \quad b = \frac{S \cdot \Delta C \cdot t_0}{t_G} \quad (2)$$

212 With  $\Delta C$  the gradient composition range,  $t_G$  the gradient time and  $t_0$  the column dead time.  
 213 In size-exclusion chromatography (SEC), solutes are ideally eluted according to their  
 214 decreasing size. When considering that the peak width is approximately constant, and that  
 215 the components are spread over the entire elution space, the peak capacity may be  
 216 expressed as Eq.(3) [40]:

$$217 \quad n_{SEC} = 1 + \left( \frac{V_0 - V_e}{V_0} \right) \frac{\sqrt{N_{measured}}}{4} \quad (3)$$

218 Where  $V_0$  is the total permeation volume (column dead volume) and,  $V_e$ , is the total  
 219 exclusion volume. The peak capacity can also be related to the total porosity,  $\varepsilon_t$ , and the  
 220 external porosity,  $\varepsilon_e$ :

$$221 \quad n_{SEC} = 1 + \left( \frac{\varepsilon_t - \varepsilon_e}{\varepsilon_t} \right) \frac{\sqrt{N_{measured}}}{4} \quad (4)$$

222 The sample peak capacity in LCxLC is theoretically the product of sample peak capacities in  
 223 each dimension:

$$224 \quad n_{2D} = {}^1n \times {}^2n \quad (5)$$

225 Where  ${}^1n$  and  ${}^2n$  are the peak capacities in first and second dimension respectively. The  
 226 superscript "1" and "2" refers to the first and second dimension respectively.

227 In LCxLC, the expected effective peak capacity is affected by three correction factors:

$$228 \quad n_{2D, effective} = \alpha(1 - \gamma) {}^1n + \alpha \cdot \beta \cdot \gamma \cdot {}^1n \cdot {}^2n \quad (6)$$

229 With  $\alpha$  correcting the peak capacity in  ${}^1D$  due to undersampling,  $\beta$  correcting the peak  
 230 capacity in  ${}^2D$  due to non-ideal transfer of the sample fraction from  ${}^1D$  to  ${}^2D$  [41], and  $\gamma$   
 231 taking into account the retention surface coverage which depends on the degree of  
 232 orthogonality between both dimensions [42]. In off-line mode,  $\alpha$  and  $\beta$  can attain values very  
 233 close to 1. This is made possible by increasing the number of fractions per peak in order to

234 reduce both under sampling (increasing  $\alpha$ ) and injection issues (increasing  $\beta$ ). The fractions  
 235 can be stored and analyzed in <sup>2</sup>D at a later time after a possible treatment (i.e. solvent  
 236 evaporation and dilution in a weaker solvent).

237 The conditions for LCxLC analysis were optimized using a home-made procedure developed  
 238 with Microsoft Excel. The calculations were based on a Pareto-optimal approach with the  
 239 aim to optimize, for a given analysis time, two conflicting criteria, minimizing the dilution  
 240 factor while maximizing the peak capacity [31].

241 The dilution factor,  $D_f$ , in dimension “i” was calculated according to the following equation:

$$242 \quad D_f = \frac{\sqrt{2\pi}}{\beta} \times \frac{{}^i\sigma_{v\,col}}{{}^iV_{inj}} \quad (7)$$

243 Where  ${}^iV_{inj}$  is the injection volume,  ${}^i\sigma_{v\,col}$  is the peak standard deviation in volume units  
 244 and can be expressed as:

$$245 \quad \sigma_{v,col} = \frac{V_0 (2.3b+1)}{2.3b\sqrt{N_{col}}} \quad (8)$$

246  $N_{col}$  was evaluated from the coefficient of the Knox equation:

$$247 \quad N_{col} = \frac{L_c}{d_p} \times \frac{1}{h(u)} \quad (9)$$

$$248 \quad \text{With } h(u) = au^{0.33} + b \frac{D_m}{ud_p} + c \frac{ud_p}{D_m} \quad (10)$$

249 With  $L_c$  the column length,  $u$  the linear velocity,  $d_p$  the particle size,  $D_m$  the diffusion  
 250 coefficient, and a, b and c, the Knox’s coefficients. The average diffusion coefficient value  
 251 was evaluated at  $1.6 \cdot 10^{-10}$  m<sup>2</sup>/s in water at 25°C, considering an average molecular mass of  
 252 4000 Da [43].

### 253 **3. Results and discussion**

#### 254 **3.1. Development of one-dimensional separations**

255 For this study, Acquity APC columns were preferred to Gel Permeation Chromatography  
 256 (GPC) columns that are most often used. These columns are based on polymer material,  
 257 while APC columns are based on silica and possess smaller dimensions. As a result, for the

258 same flow-rate, the analysis with Acquity APC columns is faster while the solvent  
259 consumption is reduced.

260 The compounds present in heavy oil matrices are within a very wide range of molecular  
261 weights. We therefore tested two different pore sizes (45 Å and 125 Å, corresponding to the  
262 APC XT 45 and APC XT 125 columns, respectively). The elution profiles obtained with these  
263 two different columns and for the three elements of interest (sulfur, vanadium, and nickel)  
264 are represented in Fig.3. With the APC XT 45 column (Fig.3a), designed for effective  
265 molecular weights ranging from 500 to 5000 g/mol, all compounds were eluted between 1.2  
266 min (exclusion volume of 960 µL) and 1.86 min (permeation volume of 1490 µL). With the  
267 APC XT 125 column (Fig.3b), designed for effective molecular weights ranging from 1000 to  
268 30000 g/mol, the compounds were better distributed over the entire separation window,  
269 between 1.2 min (960 µL) and 2.3 min (1840 µL). Very small molecules, as the suspected  
270 metalloporphyrins, were better separated on the APC XT 45 column. However, the  
271 APC XT 125 column was selected for the rest of our study for two main reasons: (i) the  
272 elution space on the APC XT 125 column was much larger (880 µL vs 530 µL) and (ii) large  
273 compounds, which were less studied than metalloporphyrins in previous reported studies,  
274 were better separated. This column was calibrated with 8 polystyrenes (PS) standards  
275 (molecular weights ranging from 266 to 44000 Da) (Fig.4). PS were detected by UV detection  
276 at 254 nm. The exclusion volume corresponding to the elution of the largest PS (44000 Da)  
277 was found to be 960 µL ( $\epsilon_e = 0.38$ ) while the total permeation volume corresponding to the  
278 elution of the smallest PS (262 Da) was found to be 1840 µL ( $\epsilon_t = 0.74$ ). The corresponding  
279 fitting curve is represented in green in Fig.4 ( $Y = -3.7928x^3 + 14.958x^2 - 21.058x +$   
280  $14.248$ ). Accordingly, it is interesting to observe by comparing Figs. 3 and 4 that the average  
281 mass can be estimated at about 4000 Da and that the petroleum sample occupies the entire  
282 elution space (from 960 to 1840 µL). As a result, considering Eq.3 (with  $h=3$ ), the maximum  
283 peak capacity value can be estimated at 18.

284 RPLC was selected as an alternative dimension because the retention mechanism is quite  
285 different from that in SEC and both may therefore lead to a high degree of orthogonality. In  
286 RPLC, the molecules are separated according to their hydrophobicity which was promising to  
287 achieve a high degree of orthogonality between both dimensions. Different stationary  
288 phases were tested by injecting the asphaltene fraction diluted in THF. The stationary phases

289 differed by the length of the grafted chain (i.e. C<sub>18</sub> or C<sub>8</sub>), by the type of the grafted chain  
290 (i.e. cyanopropyl or alkyl), by the pore size (i.e. 80 to 300 Å) and by the type of C<sub>18</sub> (i.e. hybrid  
291 silica as the Acquity BEH C18 column or polymeric grafting as the Vydac column). The  
292 different column characteristics are reported in Table S1. For Vydac C18, Zorbax-SB-CN and  
293 Hypersil Betabasic columns, a generic gradient, from 1 % to 99 % B was applied with a  
294 normalized gradient slope of 5 % (gradient slope x column dead time). For the Acquity BEH  
295 C18 column, the same gradient was used but with a normalized gradient slope of 1%.  
296 Solvent A was 80/20 ACN/H<sub>2</sub>O (v/v) and Solvent B, pure THF. The separations with specific  
297 detections for the three elements as well as with UV detection are shown in Figs.S2a, S2b,  
298 S2c and S2d for the four columns respectively. The separations are almost similar whatever  
299 the column. However, both Zorbax SB-CN and Acquity BEH C18 exhibit a bimodal separation  
300 for sulfur and vanadium. In addition, a bimodal separation also appears for nickel with  
301 Acquity BEH C18. For this reason and due to the small available column dimensions (particle  
302 size and column length, suitable for a second dimension) Acquity BEH C18 was selected for  
303 the rest of this study. Different organic solvents, including N-Methyl-2-pyrrolidone (NMP),  
304 ethyl acetate, tetrahydrofuran (THF) and acetonitrile (ACN) were tested as solvent B  
305 (100 %B). Strong solvents can lead to a total dissolution of petroleum samples and are more  
306 prone to a high recovery rate from the column. The objective was therefore to achieve a  
307 maximum recovery rate with 100 %B. Ethyl acetate was not suitable for the coupling of  
308 UHPLC with ICP-MS/MS due to its chemical incompatibility with the tubing used for the  
309 peristaltic pump. NMP was suitable in term of recovery but its high viscosity, without  
310 increasing the system temperature at more than 80 °C [44], prevents from working at high  
311 flow-rates. ACN was too weak to elute the entire sample from the column. THF was finally  
312 found to be the most relevant for dissolving the petroleum matrix as well as for being used  
313 as solvent B. A mixture of ACN and water (80/20 ACN/H<sub>2</sub>O) was selected as Solvent A in good  
314 agreement with a preceding study [21].

315 The performance of both SEC-ICP-MS/MS and RPLC-ICP-MS/MS analysis was evaluated by  
316 measuring the recovery of sulfur, vanadium, and nickel with and without column (five  
317 consecutive injections). The recovery rates for sulfur, vanadium and nickel were 104 %, 77 %, 70 %  
318 respectively in SEC while 99 %, 70 %, 67 % in RPLC. The relative standard deviations  
319 were close to 5 % in all cases. In SEC, the low recovery rate obtained for vanadium and nickel

320 suggests that a significant part of the related compounds was retained on the APC XT 125  
321 column, much likely due to adsorption mechanism on the silica surface. Similar observations  
322 for vanadium and nickel were reported by *Caumette et al.* using GPC columns on petroleum  
323 sample [22]. In contrast, sulfur compounds exhibit a very good recovery rate (close to  
324 100 %), indicating that any occurring interaction is not strong enough to result in irreversible  
325 adsorption. The recovery rates were similar in RPLC-ICP-MS/MS, supporting the idea of  
326 partial adsorption of vanadium and nickel compounds on the active silica sites while no  
327 strong adsorption exists with sulfur compounds.

328 Fig.5 shows the separations of SARA fractions in SEC-ICP-MS/MS for sulfur (Fig.5 a),  
329 vanadium (Fig.5b), and nickel (Fig.5c) and in RPLC-ICP-MS/MS for sulfur (Fig.5d), vanadium  
330 (Fig.5e), and nickel (Fig.5f). In both SEC and RPLC, the three elements were detected in the  
331 resin fraction (black chromatogram) and in the asphaltene fraction (green chromatogram). In  
332 the aromatic fraction (red chromatogram), only sulfur was detected. In the saturate fraction  
333 (blue chromatogram) a very small peak was found in SEC which was not detected in RPLC. It  
334 is important to note that the detection of nickel was difficult, especially in RPLC where the  
335 mobile phase was composed of THF but also of ACN and water.

336 In SEC, the elution profile is quite different between resin and asphaltene fractions.  
337 Asphaltenes are mostly constituted of heavy compounds or aggregates, which leads to an  
338 intense peak between 1.1 and 1.8 min. In contrast, the presence of lighter components in  
339 resins explains the intense peak at 2.3 min, close to the permeation volume, especially for  
340 vanadium (Fig.5b). A similar retention time was found for a porphyrin standard, analyzed in  
341 the same conditions, as can be observed by the overlaid dotted chromatogram in Figs.5a, 5b,  
342 and 5c. The peak at 2.3 min was therefore suspected to correspond to porphyrin  
343 compounds. By comparing Fig.5b and Fig.5c, it seems that vanadium porphyrins could be  
344 more abundant than nickel porphyrins, especially in resin fractions. This assumption will  
345 have to be confirmed in the future with quantitative analysis. Finally, as told before, column  
346 adsorption is clearly highlighted in case of asphaltenes both for vanadium and nickel which is  
347 supported by the absence of baseline return after the total permeation volume (2.3 min).  
348 Unlike other elements, sulfur is also present in aromatics with a different elution profile  
349 from those of resins and even more asphaltenes (Fig.5a), suggesting that heavy sulfur  
350 components are no more present in aromatics fraction. Asphaltenes seem to be more

351 enriched in vanadium and nickel than resins, which is in good agreement with reported  
352 studies [13].

353 As in SEC, the RPLC elution profile consists in a continuum without any isolated peak,  
354 whatever the SARA fraction. Nevertheless, a large majority of compounds is eluted with a  
355 large amount of THF, indicating that sulfur, vanadium and nickel structures are constituted  
356 of highly hydrophobic compounds, except in the aromatic fraction (red chromatogram,  
357 Fig.5d), where all compounds are eluted before 9 min. This is not surprising considering their  
358 smaller structures compared to those of the resin or asphaltene fractions. A vanadium  
359 porphyrin standard was injected separately in the same RPLC conditions (dotted  
360 chromatograms in Fig.5). Its retention time was found to be 2.6 min suggesting that  
361 porphyrin compounds were slightly retained in RPLC in these conditions. A poorly intense  
362 peak can be observed for vanadium (Fig.5e) in this retention range which could be therefore  
363 attributed to porphyrin structures. The poor intensity is mainly due to low ICP-MS/MS  
364 sensitivity in this medium (optimization performed in pure THF). It should be noticed that  
365 the elution profiles of the three considered elements are shifted towards lower retention  
366 times from asphaltenes (green chromatogram) that are more hydrophobic to aromatics (red  
367 chromatogram) that are more polar.

368 The use of SEC or RPLC alone cannot lead to the separation of one component from all the  
369 others due to the complexity of the matrix, constituted of hundreds of thousands  
370 compounds. As was observed in both techniques, the elution profile corresponds to a  
371 continuum regardless of the element. Yet, from the above results, the combination of RPLC  
372 and SEC was promising. The potential of SECxRPLC-ICP-MS/MS for the characterization of  
373 VRs samples and their fractions is discussed in the two following sections.

### 374 **3.2. Development of two-dimensional separations**

375 In this section, the potential of combining RPLC and SEC with a view to selecting the best  
376 configuration (SECxRPLC or RPLCxSEC) is theoretically evaluated using a predictive approach  
377 that takes into account the above results. Three important performance criteria were  
378 considered including (i) the effective peak capacity, (ii) the dilution factor and (iii) the  
379 analysis time. Both the off-line and the on-line modes were considered for that purpose. In

380 our quest for optimized 2D-conditions, we had to keep in mind both the instrument  
381 constraints and the available columns geometries. Those are listed below:

382 (1) In SEC, the available APC XT 125 column lengths were 3 cm, 7.5 cm, and 15 cm, always  
383 with an internal diameter of 4.6 mm. The maximum recommended flow-rate was  
384 1600  $\mu\text{L}/\text{min}$ . Due to partial adsorption, as previously discussed, the total analysis time  
385 had to be at least 40 % higher than the total permeation time for baseline return.

386 (2) In RPLC, BEH  $\text{C}_{18}$  columns are available in various lengths, various internal diameters, and  
387 various particle sizes. In this optimization study, we considered 3 different lengths (3 cm,  
388 5 cm and 15 cm), 2 different internal diameters (2.1 mm and 4.6 mm) and 2 different  
389 particle sizes (1.7  $\mu\text{m}$  and 5  $\mu\text{m}$ ).

390 (3) In order to avoid peak distortion, the maximum injected volume was found to be 1.5 %  $V_0$   
391 in SEC and 5 %  $V_0$  in RPLC ( $V_0$  being the column dead volume).

392 (4) Off-line fractionation was handmade due to non-compatibility of collector with THF. For  
393 the sake of convenience, the sampling time was therefore fixed to 10 s or higher.

394 (5) The split ratio between the first and the second dimension was not considered below  
395 1/10 to avoid too much additional band broadening due to low split ratios as previously  
396 highlighted [35].

397 Under such constraints, on-line SECxRPLC was of little interest due to only one available  
398 internal diameter for SEC columns (i.e. 4.6 mm) which made very low split ratios necessary  
399 prior to the second RPLC dimension operated with usual UHPLC columns (i.d. 2.1 mm).  
400 Indeed, it was shown that such low split ratios (<1/5-1/10) can lead to a significant additional  
401 peak broadening [35], thereby substantially decreasing the effective peak capacity. Similarly,  
402 off-line RPLCxSEC was also not possible because in that case, a very large number of  
403 fractions should be collected which was beyond the capacity of the vial rack. Furthermore,  
404 the mobile phase was little compatible with the RPLC mobile phase composed partly of THF.  
405 Consequently, our choice was made between on-line RPLCxSEC and off-line SECxRPLC.

406 In off-line LCxLC the two dimensions are disconnected. As a result, the peak capacity in each  
407 dimension is only limited by the maximum column length that can be used considering the  
408 maximum pressure delivered by the instrument and withstood by the column. Thus, in case  
409 of two fully orthogonal LC-systems, the peak capacity in off-line LCxLC can theoretically be  
410 quite impressive. However considering the resulting huge number of runs and hence the

411 very long analysis time and the high associated dilution, a good trade-off has to be found  
412 between a high separation power, a reasonable analysis time and a good sensitivity. We  
413 therefore focused on the same total analysis time of about 150 min, no matter it was off-line  
414 SECxRPLC or on-line RPLCxSEC.

415 Our first choice was on-line RPLCxSEC. This configuration seemed to be very attractive  
416 because the second dimension is not performed in gradient elution but in pure THF.  
417 Optimized values for ICP-MS/MS parameters are fully dependent on the mobile phase  
418 composition. As a result, once they have been optimized for a given composition (e.g.  
419 100 %THF), they may be inappropriate for compounds eluting during the gradient run in a  
420 mixture composed of water and acetonitrile in addition to THF. Furthermore, we found that  
421 the the signal intensity was higher with pure THF than with any mixture of ACN/water/THF.  
422 The same pareto-optimality approach as the one previously developed [31,32] was applied  
423 to search for optimized conditions in on-line RPLCxSEC, considering a gradient time of 150  
424 min in the first dimension. This was achieved by varying <sup>1</sup>D column length (15; 5 and 3 cm),  
425 <sup>1</sup>D particle size (5 and 1.7 μm) <sup>1</sup>D column internal diameter (4.6 and 2.1 mm), <sup>1</sup>D flow-rate  
426 (from 10 to 500 μL/min), <sup>1</sup>D split ratio (from 1 down to 1/10), <sup>2</sup>D column length (15; 7.5 and  
427 3 cm), <sup>2</sup>D column temperature (30 and 60 °C), <sup>2</sup>D flow-rate (from 800 to 1600 μL/min) and  
428 the sampling rate (from 1 to 3 fractions per peak). The resulting pareto plots, considering a  
429 full occupation of the retention space ( $\gamma=1$ ) are shown in Fig.6. On-line mode usually involves  
430 the use of short second dimension columns. In our case, only a very short SEC column (i.e.  
431 3 cm) operated at the maximum allowed flow-rate (i.e. 1600 μL/min) and at the maximum  
432 allowed temperature (i.e. 60 °C) provided enough time to carry out the separation. Among  
433 all the different sets of conditions represented by red triangles in Fig.6, some of them,  
434 located on the pareto-optimal front (red dotted line), are of prime interest. Conditions and  
435 performance of optimized settings numbered #1 to #5, are detailed in Table 1. Setting #1 is  
436 expected to provide the highest effective peak capacity (1700), despite a high dilution factor  
437 (i.e. 25) whereas setting #5 should lead to the lowest peak capacity (1000) but with a more  
438 satisfactory dilution factor (i.e. 3). It is interesting to note that for the 5 sets of optimized  
439 conditions, only a column internal diameter of 2.1 mm seems to be appropriate in <sup>1</sup>D.  
440 Increasing <sup>1</sup>D column length has a negative impact on effective peak capacity while a positive  
441 one on dilution. As shown by the results listed in Table 1, the number of runs in the second



442 dimension is quite impressive (300 to 450). Moreover, the split ratio is 1/5 or lower, which  
443 could result in a decrease in the effective peak capacity due to additional peak broadening. It  
444 should also be noted that an additional issue with SEC in the second dimension was raised by  
445 Reingruber et al. [45]. The injection solvent in SEC (i.e. the first dimension mobile phase), is  
446 composed of a mixture of ACN, water, and THF, and hence is prone to produce adsorption  
447 phenomena in SEC and hence significant peak tailing.

448 Our second choice was off-line SECxRPLC. The conditions were imposed by the constraints  
449 we had to deal with. The best set of conditions, considering the necessity of fractionating  
450 every 10 s, is represented by a blue circle in Fig.6. The related conditions are listed in Table  
451 2. 12 fractions were collected between 1 and 2 min. The gradient time in the second  
452 dimension was set at 10.2 min so that the total analysis time was close to 150 min. Under  
453 such conditions, the calculated effective peak capacity is 2600, much higher than in on-line  
454 RPLC x SEC (<1700) whereas the dilution factor is quite similar to the one calculated for  
455 setting #1 (27 vs 25).

456 Considering the huge number of runs in on-line RPLCxSEC without any significant gain in  
457 effective peak capacity or in sensitivity compared to off-line SECxRPLC, on line RPLCxSEC was  
458 abandoned and off-line SECxRPLC was definitely selected to analyze complex samples  
459 coming from the petroleum industry.

### 460 **3.3. Analysis of the SARA fractions by SECxRPLC-ICP-MS/MS**

461 SARA fractions were analyzed by off-line SECxRPLC-ICP-MS/MS with conditions listed in  
462 Table 2. Fig.7 shows the 2D-contour plots for the fractions of aromatics, resins and  
463 asphaltenes considering the sulfur and vanadium elements. As told before, this 2D-set-up  
464 associates hydrophobicity property to mass property. Large compounds, early eluted in SEC  
465 are strongly retained in RPLC, likely due to the presence of a large number of carbons.  
466 Unfortunately, too much dilution in LCxLC (as above discussed) prevented from detecting  
467 nickel, present at a very low level. For the same reason, vanadium could not be detected in  
468 the aromatic fraction. 2D-contour plots are significantly different depending on both the  
469 fraction and the element. For the asphaltene fraction, in case of both sulfur (Fig.7c) and  
470 vanadium (Fig.7f), whereas there is no baseline separation in SEC, a bimodal distribution  
471 with baseline separation appears in RPLC for the most retained compounds. This suggests

472 that both sulfur and vanadium compounds are present in two different families differing by  
473 their hydrophobicity. Structural identification by NMR as well as LCxLC-MS analysis should be  
474 carried out to get additional information on these compounds, which should be of great  
475 interest for the petroleum industry.

476 In order to underline the main differences between the SARA fractions, the contours of the  
477 2D-contour plots are overlaid for sulfur (Fig.8a) and for vanadium (Fig.8b). As can be seen,  
478 each SARA fraction has its own contour, with a form different depending on the fraction. In  
479 case of sulfur as example (Fig.8a), the form related to aromatics suggests the presence of  
480 lighter compounds compared to resins and asphaltenes. It can also be noted that the  
481 contour of resins is included into the one of asphaltenes both in case of sulfur and vanadium.  
482 The peak present at the top-left of Fig.8e is noticeable. It is likely due to porphyrin  
483 structures, widely present in the asphaltene fraction. Such contours obtained by SECxRPLC-  
484 ICP-MS/MS could be very useful in the future to compare the fractionation performance of  
485 SARA process which may vary from one laboratory to another.

#### 486 **4. Conclusion**

487 The objective of this work was to develop a two-dimensional separation method, coupled to  
488 elemental detection, for analyzing complex petroleum samples. A high separation power  
489 being mandatory for such complex samples, we first evaluated the potential of one-  
490 dimensional separation (SEC or RPLC), and then we pointed out that 2D-separations allowed  
491 to achieve much higher peak capacities but at the cost of higher dilution. An effective peak  
492 capacity of 2600 could indeed be achieved in less than 2.5 h. Both off-line and on-line LCxLC  
493 separations were compared using a pareto-optimality approach with the aim of maximizing  
494 the peak capacity while minimizing the dilution factor in a given analysis time. The best 2D-  
495 conditions in term of peak capacity were found in off-line SEC x RPLC, also considering the  
496 huge number of runs required in on-line RPLC x SEC.

497 To the best of our knowledge, such combination of SEC and RPLC, operated in a  
498 comprehensive off-line mode and hyphenated to ICP-MS/MS, is the most advanced tool,  
499 without any equivalent in the literature, for the speciation of vanadium, nickel and sulfur in  
500 petroleum samples.

501 SEC as first dimension allows the separation according to the molecular weight of the  
502 analytes within a range of 1000 to 30000 g/mol. RPLC as second dimension allows the  
503 separation of the molecules according to their hydrophobicity making this coupling very  
504 attractive. By coupling these two different retention mechanisms, the species contained in  
505 petroleum fractions could be distributed over the entire elution space. This 2D-method  
506 hyphenated to ICP-MS/MS could achieve the speciation of vanadium, sulfur and nickel  
507 within a single run.

508 The best 2D-conditions were applied to the characterization of SARA fractions. The metal  
509 distribution shows that larger compounds (short retention times in SEC) are more  
510 hydrophobic (high retention times in RPLC). Whereas heavy masses are not well separated in  
511 SEC alone, they are partly separated in RPLC making the combination of both techniques of  
512 great interest.

513 In the future, it could be possible to apply this analysis method to more complex samples,  
514 such as non-fractionated petroleum samples coming from refining units.

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683

## 684 **Figure Captions**

685 Figure 1: Scheme of the SARA fractionation of a vacuum residue (VR).

686

687 Figure 2: Schematic representation of the instrument setup in off-line SECxRPLC-ICP-MS/MS  
688 (tubing dimensions are given in the experimental section).

689

690 Figure 3: Overlaid separations of an atmospheric residue (AR Venezuela) in SEC-ICP-MS/MS  
691 with specific detection of sulfur, vanadium and nickel. Conditions: (a) APC XT 45 column  
692 (150 x 4.6 mm, 1.7  $\mu\text{m}$ ) and (b) APC XT 125 column (150 x 4.6 mm, 2.5  $\mu\text{m}$ ). Other conditions  
693 given in the experimental section.

694

695 Figure 4: Separation of 8 polystyrene standards (contained in 2 different solutions) in SEC-UV  
696 (254 nm). The green dotted curve represents the SEC calibration curve. Conditions: APC XT  
697 125 column (150 x 4.6 mm, 2.5  $\mu\text{m}$ ). 5  $\mu\text{L}$  injected. Other conditions given in the  
698 experimental section.

699

700 Figure 5: Overlaid separations of SARA fractions in (a, b, c) SEC-ICP-MS/MS and in (d, e, f)  
701 RPLC-ICP-MS/MS with specific detection of (a, d) Sulfur, (b, e) Vanadium and (c, f) Nickel. SEC  
702 conditions: APC XT 125 (150 x 4.6 mm, 2.5  $\mu\text{m}$ ) column; Mobile phase: THF. 0.8 mL/min;  
703 30 °C; 15  $\mu\text{L}$  injected. RPLC conditions: Acquity BEH C18 (50 x 2.1 mm, 1.7  $\mu\text{m}$ ) column; A:  
704 80/20 ACN/H<sub>2</sub>O; B: THF; 1 % B to 99 % B in 15.2 min; 0.8 mL/min; 30 °C; 5  $\mu\text{L}$  injected. Flow  
705 splitter prior to ICP-MS/MS (1:1). Peak of 2,3,7,8,12,13,17,18-Octaethyl-21H,23H-porphin in  
706 grey dotted line.

707

708 Figure 6: Full pareto plots (red triangles) of dilution factor versus effective peak capacity for  
709 an analysis time of 150 min in on-line RPLCxSEC ( $\gamma=1$ ). The pareto-optimal front is  
710 represented in red dotted line. The conditions of setting #1 to #5 are listed in Table 1. The



711 blue circle corresponds to the optimized set of conditions in off-line SECxRPLC (listed in Table  
712 2). See text for more explanations.

713

714 Figure 7: SECxRPLC-ICP-MS/MS analysis of SARA fractions with specific detection, of (a, b, c)  
715 Sulfur and (d, e, f) Vanadium: (a, d) aromatic, (b, e) resin and (c, f) asphaltene fractions. See  
716 conditions in Table 2.

717

718 Figure 8: Overlay of the SECxRPLC-contours of aromatic (green, dotted line), resins (blue,  
719 solid line) and asphaltene (red, dashed line) fractions with ICP-MS/MS specific detection of  
720 (a) Sulfur and (b) Vanadium. See conditions in Table 2.

Figure 1

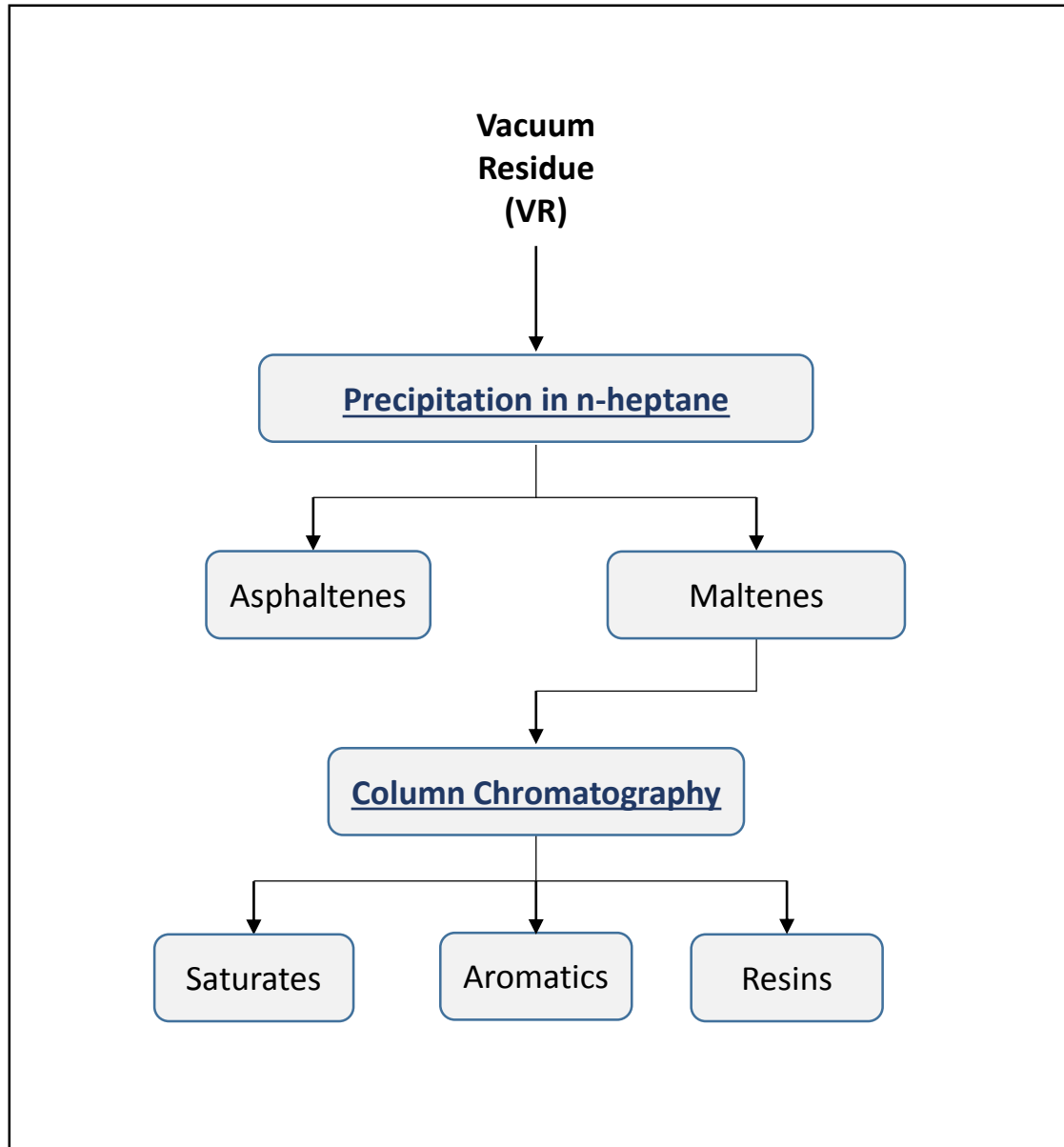


Figure 2

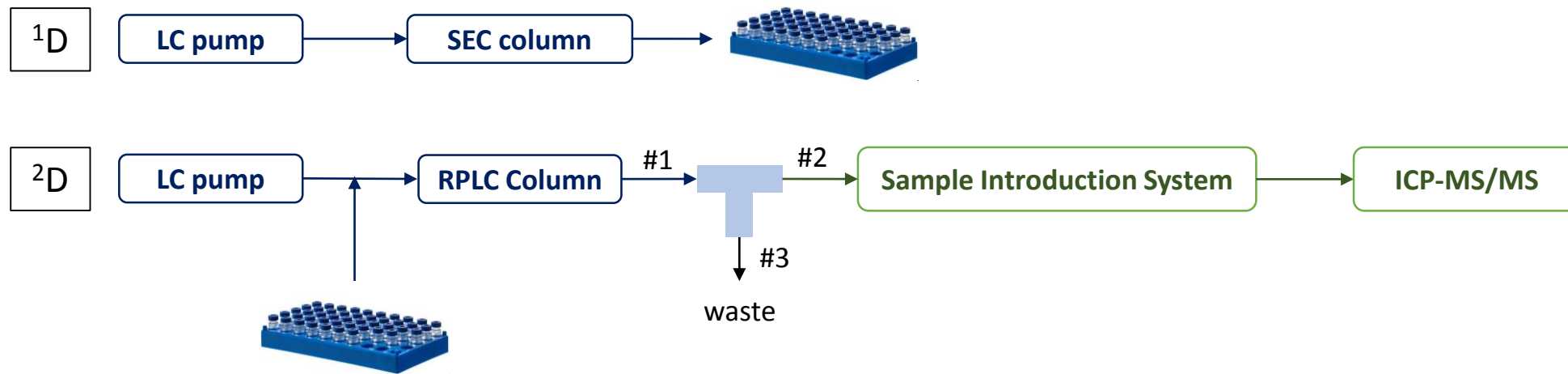


Figure 3

— Sulfur      — Vanadium      — Nickel

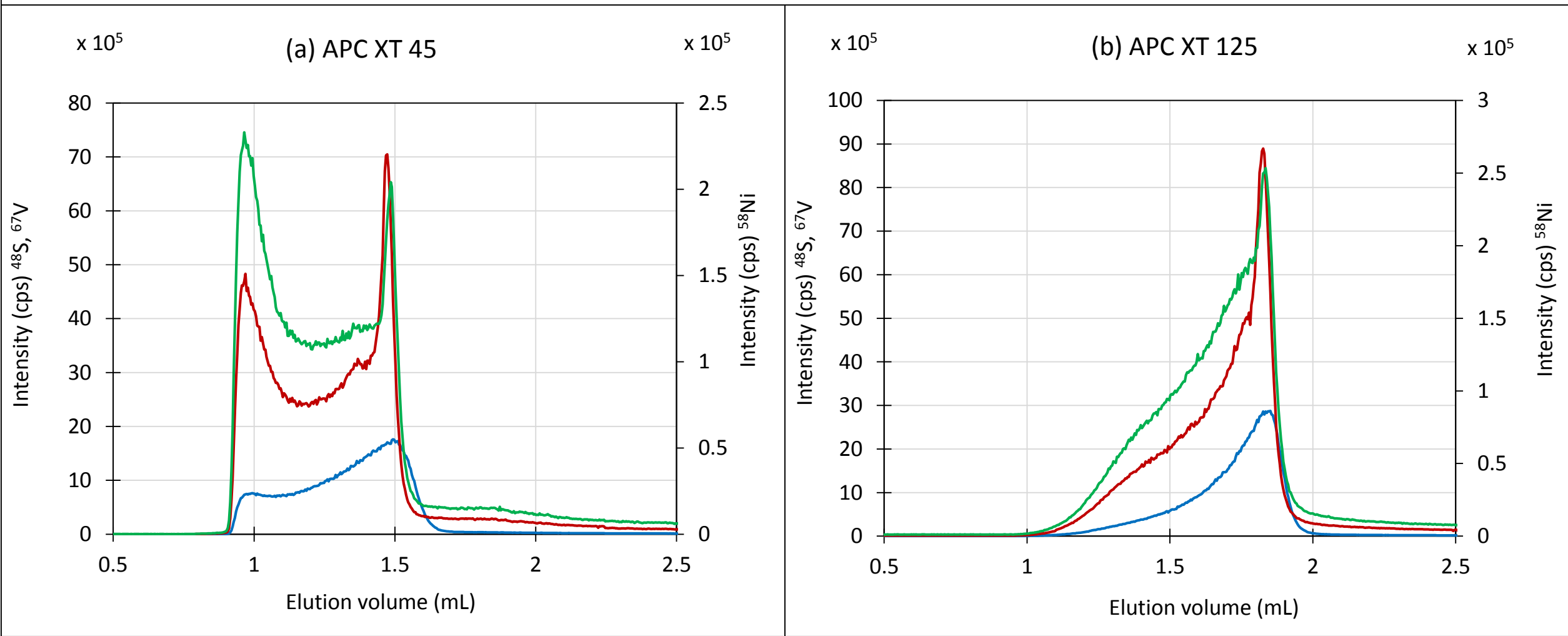


Figure 4

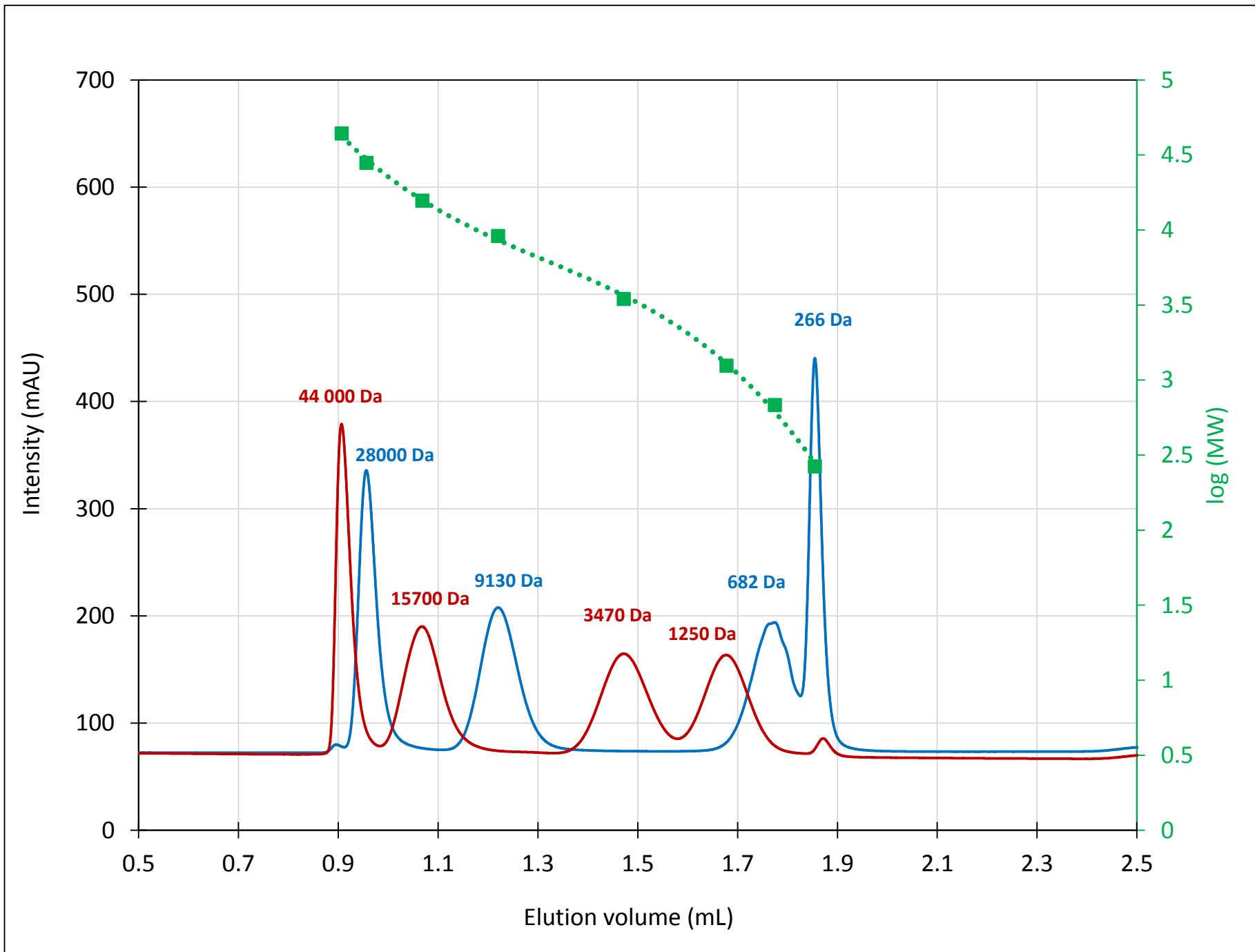


Figure 5

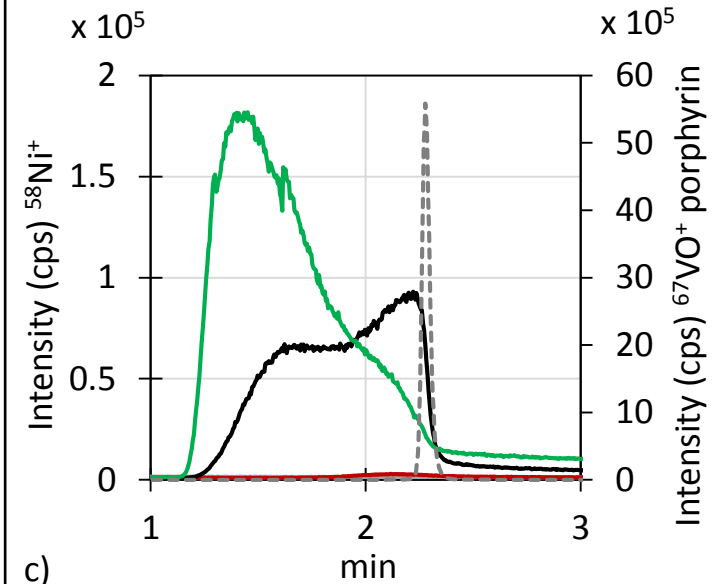
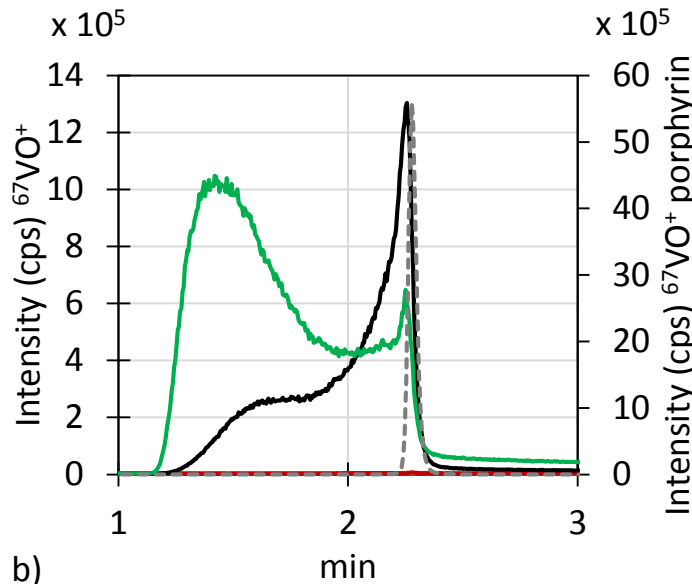
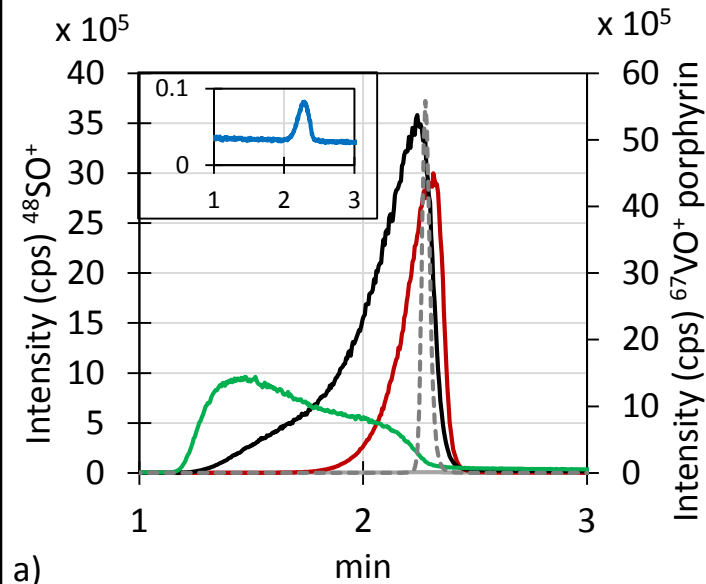
— Saturates    — Aromatics    — Resins    — Asphaltenes    ····· Standard porphyrin

**Sulfur**

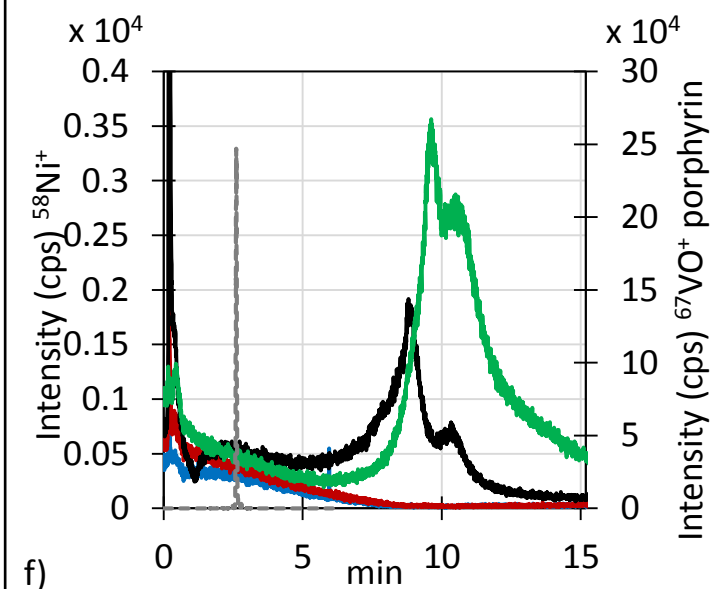
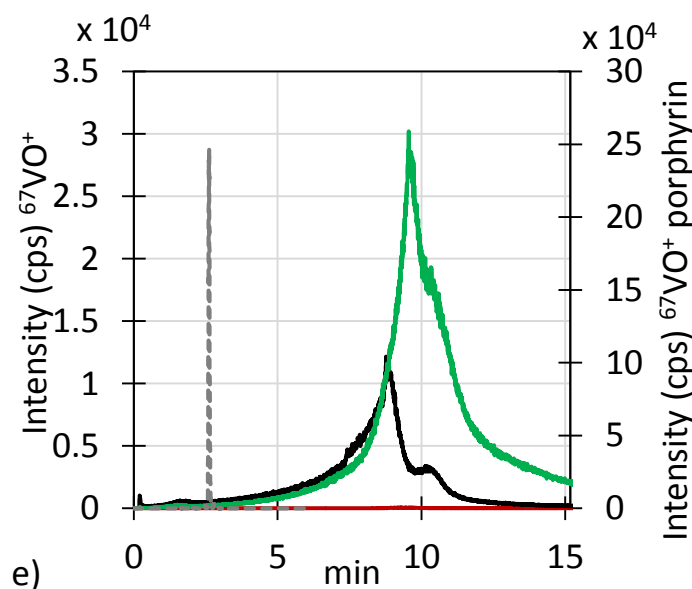
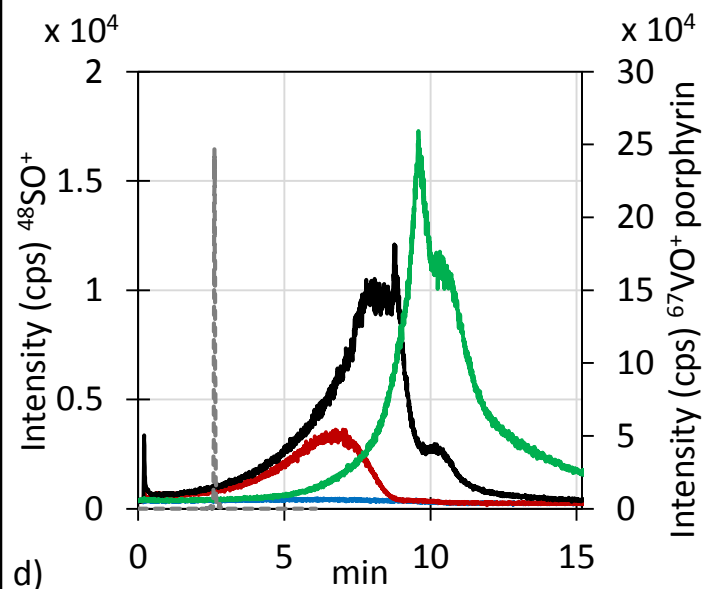
**Vanadium**

**Nickel**

**SEC-ICP-MS/MS**



**RPLC-ICP-MS/MS**



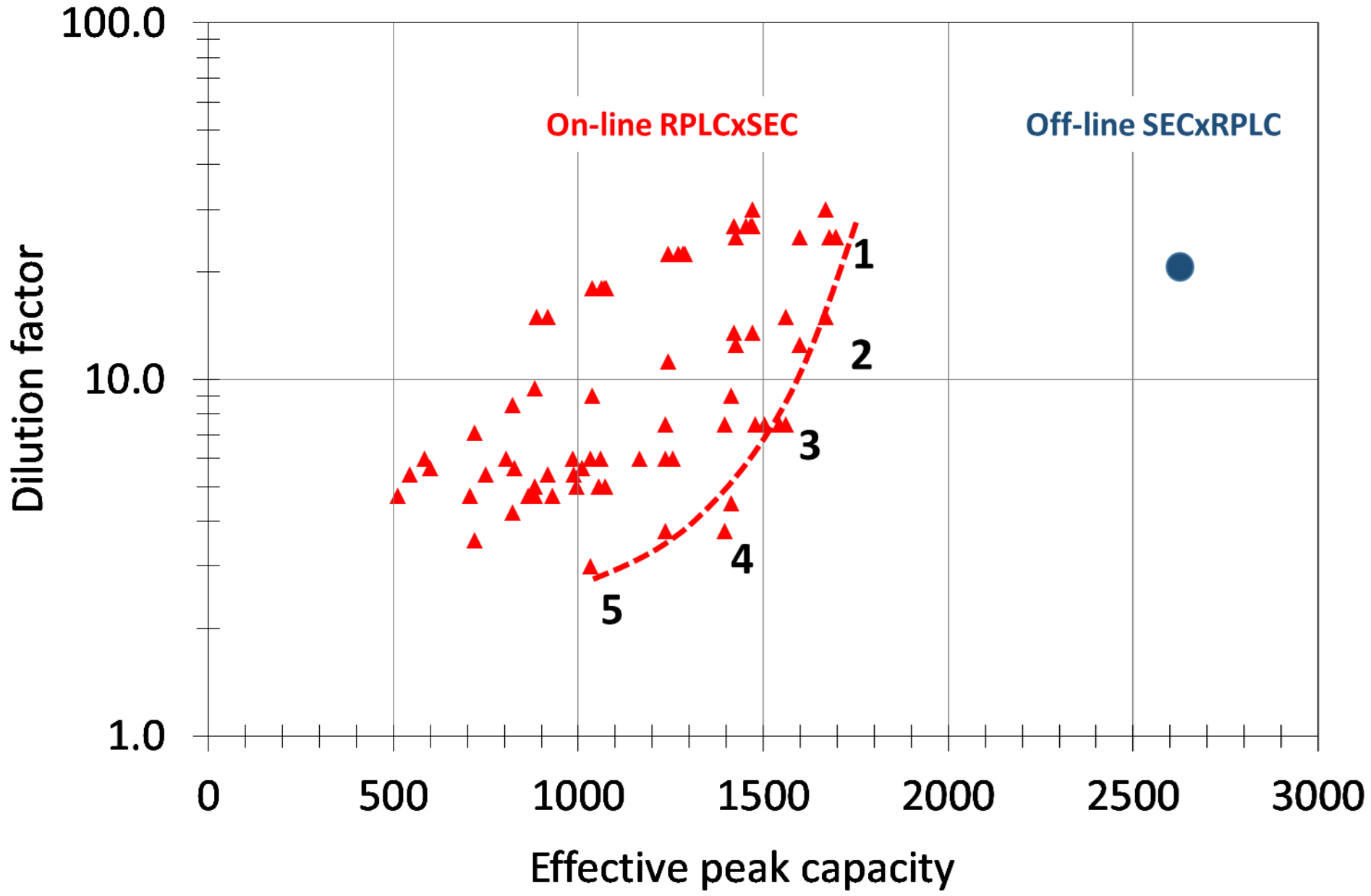


Figure 6

Figure 7

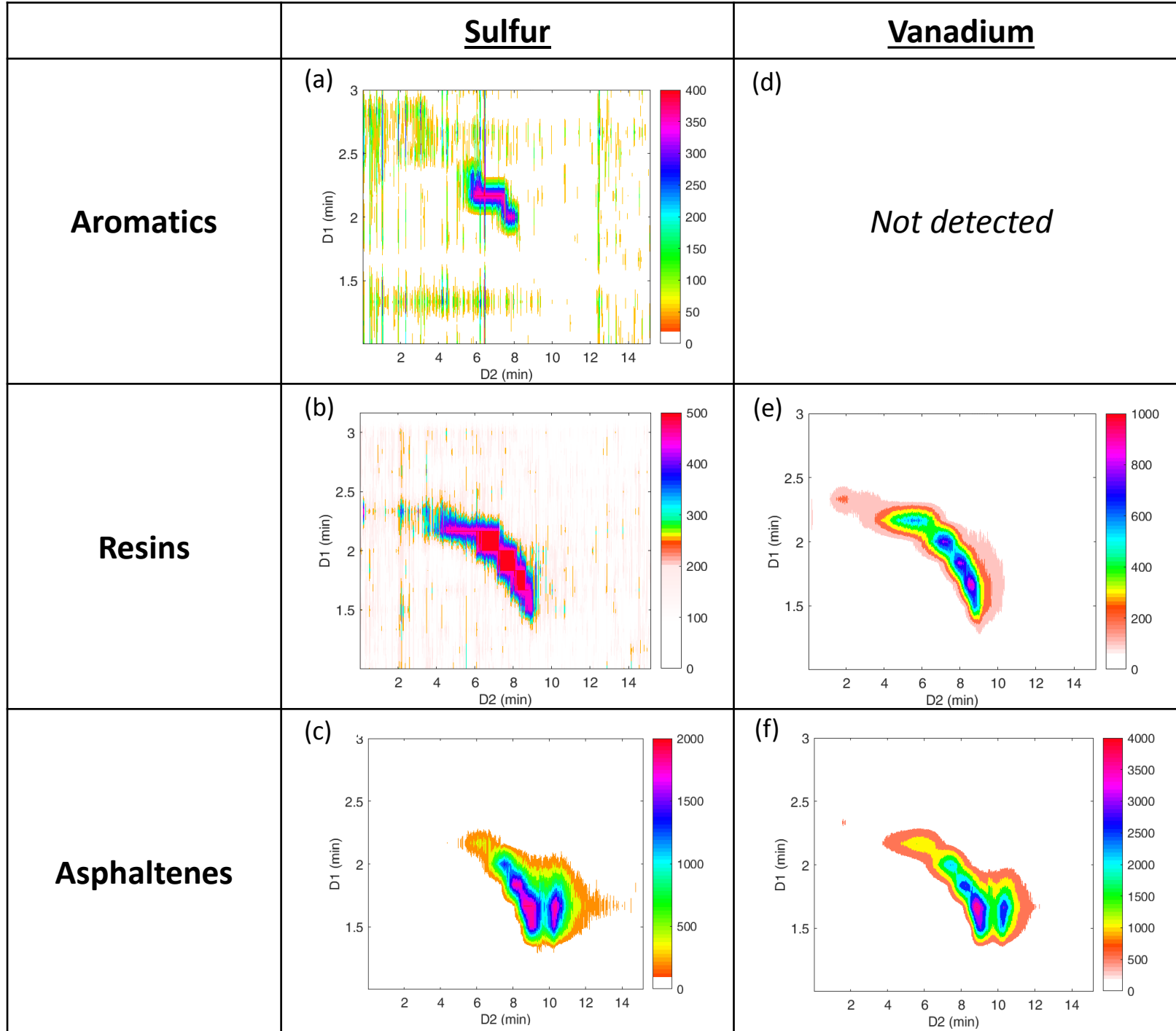
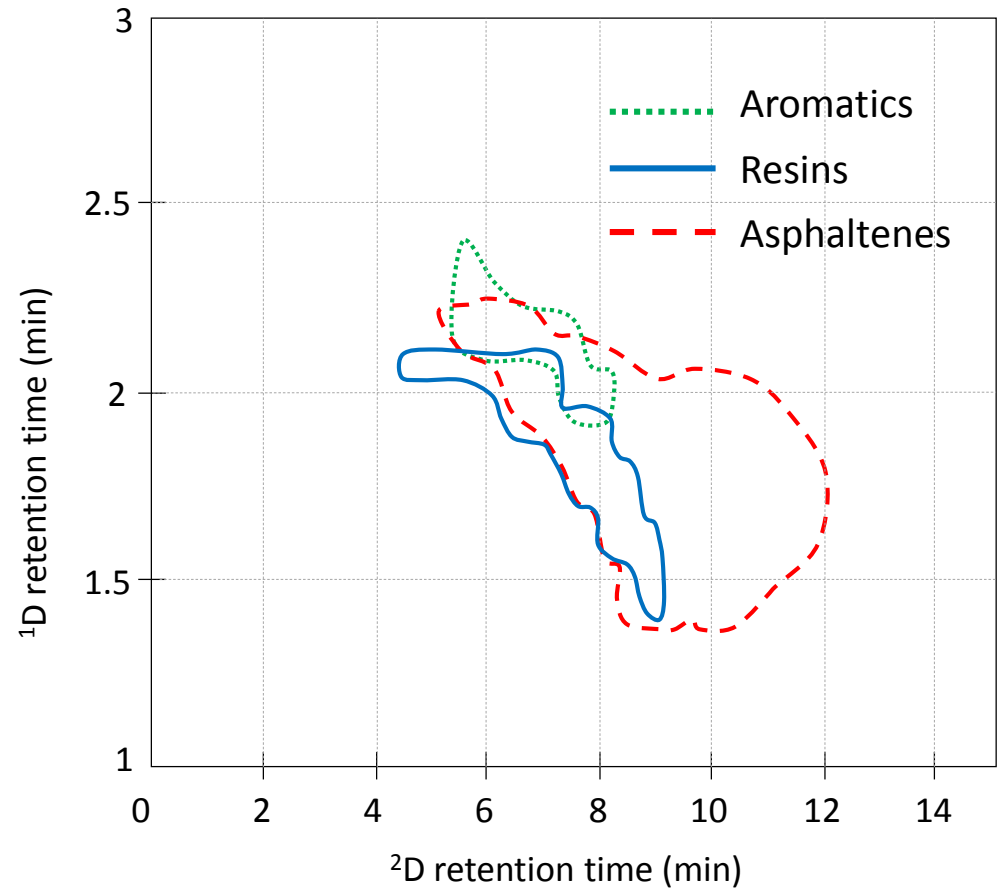




Figure 8

(a) Sulfur



(b) Vanadium

