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1 **Suitable interface for coupling liquid chromatography to inductively coupled**
2 **plasma-mass spectrometry for the analysis of organic matrices. 2 Comparison**
3 **of sample introduction systems**

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11 **Abstract**

12 Liquid chromatography (LC) coupled with a specific detection such as inductively coupled
13 plasma-mass spectrometry (ICP-MS/MS) is a technique of choice for elementary speciation
14 analysis. The analysis of organic matrices requires the introduction of volatile solvents into
15 the plasma which is an analytical challenge for this coupling technique. A large number of
16 instrumental limitations may contribute to considerably reduce the expected sensitivity.
17 Among those, we were interested in the solute dispersion into the interface located
18 between LC and ICP-MS. This interface consists in both a Sample Introduction System (SIS)
19 and a possible flow splitter. This study, divided into two parts, investigated the analytical
20 performance (in terms of sensitivity and efficiency) generated by the coupling of LC and ICP-
21 MS in the specific case of organic matrices. In Part I [1], we previously discussed the impact
22 of extra column dispersion on the performance of LC-ICP-MS, first from a theoretical point of
23 view and next, from a study of 55 published results in LC-ICP-MS. It was shown that SIS was
24 rarely optimized with respect to its contribution to extra-column band broadening. The
25 critical impact of flow splitting on extra-column dispersion was also pointed out. The present
26 Part II is dedicated to the comparison of commercially available SIS by assessing their
27 contribution to the loss in both efficiency and sensitivity resulting from extra-column band
28 broadening. It is shown that the peak variance, due to SIS, can vary from 10 to 8000 μL^2
29 depending on the combination of both nebulizer and spray chamber. Whereas the highest

30 values (i.e. $> 2000 \mu\text{L}^2$) are much too high in high performance liquid chromatography
31 (HPLC), even the lowest values (i.e. $< 100 \mu\text{L}^2$) can be inappropriate in ultra-high pressure
32 liquid chromatography (UHPLC) as highlighted in this study. Moreover, in light of these
33 results, it appears that the peak variance depends on key parameters including the geometry
34 of SIS devices and the flow rate entering the interface.

35 **Keyword**

36 Speciation – Ultra high performance Liquid chromatography - Inductively coupled plasma -
37 Extra-column dispersion - Sample Introduction System - LC-ICP-MS

38 **1. Introduction**

39 Liquid Chromatography (LC) hyphenated to a specific detection such as Inductively
40 Coupled Plasma-Mass Spectrometry (ICP-MS/MS) is widely used to obtained elemental
41 information and to discriminate species in a given matrix [2,3]. Reverse Phase Liquid
42 Chromatography (RPLC) [4] and Size-Exclusion Chromatography (SEC) [5,6] both performed
43 with large amounts of organic solvent in the mobile phase are gaining interest, as previously
44 discussed in Part I. Over the past few years, LC techniques have been extensively used for
45 speciation analysis, in particular with narrow bore (2.1 mm i.d.) or microbore (1 mm i.d.)
46 columns, packed with small particles (sub $2 \mu\text{m}$) and operated under ultra-high pressure
47 (UHPLC) in order to obtain high column plate number within short analysis times [7]. In
48 UHPLC (i.e. 2.1 mm i.d. column packed with sub- $2 \mu\text{m}$ particles), the optimal flow-rate is
49 usually lower than in conventional HPLC (i.e. 4.6 mm i.d. column packed with $5 \mu\text{m}$ particles),
50 which is advantageous when using organic solvent in ICP-MS. If the flow rate entering the
51 plasma source is decreased, the amount of organic solvent is also decreased, thereby
52 improving the plasma stability [8]. Some key issues have to be considered for coupling LC to
53 ICP-MS in case of organic matrices. Those are thoroughly detailed in the first part of this
54 study [1]. The interface between LC and ICP-MS is made of a Sample Introduction System
55 (SIS) and a possible flow splitter prior to SIS which may be required in case of organic
56 matrices, to reduce the amount of organic solvent reaching the plasma [8]. As highlighted in
57 Part I, various SIS have been used over the past decades for the speciation analysis of
58 organic matrices but suitable choices were not always made. Moreover, our previous study

59 clearly showed that using flow splitting to reduce the flow-rate entering SIS or any detection
60 device could result in a significant increase in band broadening, especially when the split
61 ratio is low.

62 The success of highly efficient separations depends on both the intrinsic column
63 efficiency and the ability to preserve it by minimizing extra-column band broadening [9]. In
64 order to keep column efficiency, it is of prime importance to reduce extra-column band
65 broadening in each device of the LC-ICP-MS instrument and in particular, in the interface
66 prior to ICP-MS. This issue is especially critical in case of UHPLC separations because of low
67 peak volumes. For complex samples, on-line two-dimensional liquid chromatography (2D-LC)
68 is known to be a powerful separation technique [10]. However, due to the extreme thinness
69 of second dimension peaks, the success of this technique strongly depends on the reduction
70 of the volumes located between the second dimension column and the detector [9].
71 Considering solute dispersion, the aim of this study was to compare the commercially
72 available devices (six nebulizers along with nine spray chambers) and their resulting
73 combinations (i.e. 31) that are most commonly used [1]. Some recommendations for a
74 proper selection of both nebulizer and spray chamber are provided as a result of this study.

75 **2. Experimental section**

76 **2.1 ICP-MS/MS instrument and conditions**

77 As shown in Part I, a large panel of nebulizers and spray chambers can be found in the
78 literature, with different designs. In this work, concentric, parallel path, and cross-flow
79 nebulizers were compared and evaluated, as well as, several spray chambers, such as single
80 pass, double pass, impact bead, cyclonic. The experiments were carried out using an
81 Agilent 8800 ICP-MS/MS system (Agilent Technologies). For the purpose of the study, 31
82 different SIS configurations, each composed of one nebulizer (among 6) and a spray chamber
83 (among 9) were tested. Among 54 possible combinations, 31 were achievable from an
84 instrumental point of view (some nebulizers cannot be mounted on every spray chamber
85 due to instrumentation issues). A detailed description of the six nebulizers and the nine
86 spray chambers are given in Table 1 and Table 2 respectively. Some spray chambers were
87 equipped with a temperature controlled device and some of them were equipped with a
88 baffle as highlighted in Table 2.

89 The instrument was equipped with a torch with a 1 mm i.d. injector (Agilent
90 Technologies). The analyzer unit consists of two quadrupoles mass analyzers and an
91 octopole collision-reaction cell placed between both quadrupoles. Vanadium was detected
92 in MS/MS mode with oxygen as a reaction gas in the cell. Vanadium 51 was shifted to mass
93 67. The following ICP-MS/MS parameters were used: plasma power: 1500 W, auxiliary gas:
94 0.9 L/min, plasma gas flow rate: 15 L/min. Both sampler and skimmer cones were made with
95 platinum instead of nickel as usual, due to platinum inertness. The experimental conditions
96 for the 31 tested combinations of a nebulizer and a spray chamber are listed in Table 3. For
97 parameter optimization, a tune solution (1 ppb Li, Y, Tl, Ce, Co) was continuously delivered
98 to the nebulizer using a peristaltic pump equipped with Viton® tubing dedicated to organic
99 solvents at a speed of 0.1 rps (corresponding to a flow rate of 400 µL/min) except for total
100 consumption spray chamber (i.e. 0.05 rps equivalent to 200 µL/min). For transient signal, the
101 integration time was set at 0.1 s. ICP-MS/MS parameters were optimized to have the best
102 trade-off between a high intensity for standard solutions of lithium, yttrium, and thallium at
103 1 ppb level and a low oxide ratio. The ratio $^{156}\text{CeO}^+ / ^{140}\text{Ce}^+$ was used to monitor the oxide
104 ratio level which should be kept below 10 % if possible. The plasma gas flow rate, the
105 auxiliary gas flow rate, the radio-frequency power, and the radiofrequency matching were
106 kept constant for all studied combinations of nebulizers and spray chambers. The carrier gas
107 flow rate, the sampling depth, and the optional gas flow rate were optimized to achieve the
108 higher intensity (cps) for Li, Y, and Tl. In order to ensure a stable plasma and to avoid carbon
109 deposit on the cone surface (sampler and skimmer), oxygen was mixed with argon (20 % of
110 O₂ in Ar) and carried to ICP-MS/MS with HMI tubing. The amount of oxygen was optimized
111 by monitoring carbon emission band when introducing organic solvent, by visually assessing
112 that no carbon was deposited on the sampler cone. Then, the carrier gas was optimized in
113 the range of 0.3 to 0.9 L/min for every system. The intensity of Li, Y and Tl was monitor while
114 changing carrier gas value. Such optimization was performed for every combination of
115 nebulizers and spray chambers (#1 to #31) as shown in Table 3. An example of the carrier gas
116 optimization is shown in supplementary information Fig.S1.

117 **2.2 UHPLC instrument conditions**

118 Flow injection analysis was performed with an ACQUITY UPLC I-Class system (Waters,
119 Milford, MA, USA), including a high-pressure binary solvent pump with a maximum delivery

120 flow-rate of 2 mL/min, an autosampler with a flow-through needle of 15 μ L, a column oven
121 with a maximum temperature of 90°C and a diode array detector with a 500 nL flow-cell
122 withstanding pressure up to 70 bar. The injection volume was 1 μ L. The wavelength was set
123 at 407 nm for porphyrin detection. Data acquisition with a sampling rate of 40 Hz and
124 instrument control was performed by Empower software. All experiments were performed
125 with acetonitrile as mobile phase at 400 μ L/min (except for Total consumption spray
126 chamber working at 200 μ L/min), using a zero-dead volume union in place of the column.
127 The oven temperature was 30 °C. The DAD detector was connected to the nebulizer with a
128 PEEK tubing. Its dimensions were selected depending on the spray chamber design, namely
129 67 cm x 65 μ m for Agilent 8800, Spectro Large and Single-pass and 111 cm x 100 μ m for
130 Twinnabar, Impact bead, Spectro Small, PC³, IsoMist, and Total consumption.

131 2.3. Chemicals and sample preparation

132 2,3,7,8,12,13,17,18-Octaethyl-21H,23H-porphine vanadium(IV) and LC-MS grade
133 acetonitrile (ACN) were purchased from Sigma-Aldrich (Steinheim, Germany).
134 Tetrahydrofuran (unstabilized) UHPLC/MS grade, obtained from Biosolve Chimie SARL
135 (Dieuze, France) was used as sample solvent. SPEX CertiPrep (Metuchen, NJ, USA) Co, Ce, Y,
136 Tl, Li monoelemental standards in 2% HNO₃ (1000 μ g/L) were used to prepare the
137 multielement standard solution used for calibration. This solution with 1 ppb Co, Ce, Y, Tl, Li
138 in acetonitrile was used for daily calibration of the ICP-MS/MS. Porphyrin sample was
139 prepared at a concentration of 10 ppm in THF. Solutions were stored at 5 °C.

140 3. Results and discussion

141 3.1. Comparison of 31 commercially available interfaces

142 Extra-column solute dispersion was evaluated in flow injection analysis by injecting a
143 sample of porphyrin, using two different detectors (i.e. UV and ICP-MS/MS) in series and a
144 SIS interface between them. A zero-dead volume union was used in place of the column.
145 Porphyrin was first detected by UV, and then by ICP-MS/MS at $m/z = 67$. This compound was
146 found to be attractive because of the presence of chromophores for UV detection and of
147 chelated metal for ICP-MS/MS detection. The variance of dispersion due to the SIS interface
148 (σ_{SIS}^2) was estimated by subtracting the peak variance, resulting from the solute travel into

149 the UHPLC/UV instrument ($\sigma_{ext,UHPLC/UV}^2$), to the total peak variance, resulting from the
150 whole solute travel ($\sigma_{ext,total}^2$):

$$151 \quad \sigma_{SIS}^2 = \sigma_{ext,total}^2 - \sigma_{ext,UHPLC/UV}^2 \quad (1)$$

152 The calculations of σ_{SIS}^2 were based on the assumption that the contribution of both
153 zero-dead volume union and ICP-MS/MS analyzer to total solute dispersion could be
154 considered as not significant and hence could be disregarded.

155 Due to severe peak distortions, especially due to SIS, peak variances in volume unit, σ_v^2 ,
156 were calculated by means of the second order central moment, using an in-house program.

157 σ_{SIS}^2 values resulting from solute dispersion in 31 different combinations of nebulizers
158 and spray chambers (31 settings as numbered in Table 3) were determined according to
159 Eq.1. To ensure a good reliability of the results, the solute was injected five consecutive
160 times.

161 Among the 31 different SIS interfaces that were assessed with respect to extra-column
162 band broadening, PC³, Twinnabar, and IsoMist are the most reported spray chambers in the
163 literature [1]. The peak variances was determined without column for five replicates. Mean
164 variance values in volume units and the corresponding Relative Standard Deviations (RSD)
165 were calculated for each of the 31 SIS interfaces alone and for the UHPLC/UV system alone.
166 Values for SIS alone (σ_{SIS}^2 according to Eq.1) are listed in Table 4. The mean variance value
167 for the UHPLC/UV system ($\sigma_{ext,UHPLC/UV}^2$) was about 8 μL^2 . This value was found to be quite
168 repeatable (RSD < 5%), thereby highlighting the reliability of the calculation method. As can
169 be seen in Table 4, the range of σ_{SIS}^2 values, depending on SIS, is very large (from 6 to
170 8070 μL^2). Unlike UHPLC/UV system, the determination of σ_{SIS}^2 was poorly repeatable for
171 most SIS with RSD values sometimes higher than 25%. For SIS #23, the RSD value was even
172 close to 60 % which might arise from the design of the spray chamber wherein the aerosol is
173 not sorted out and entirely sent to the plasma, making solute dispersion uneven. More
174 generally, solute dispersion in SIS appears to be less repeatable than in the rest of the
175 UHPLC-UV/ICP-MS/MS system, suggesting a more uncertain dispersion process.

176 The lowest peak variance (i.e. 6 μL^2) was found with Twinnabar as spray chamber and PFA-LC
177 as nebulizer (SIS #3 in Table 4). Different SIS combining Twinnabar and different nebulizers

178 are compared in Fig.1. The studied nebulizers included concentric (Opalmist, Savillex,
179 MicroMist, and PFA-LC) and parallel path (Burgener) ones. The peaks shown in Fig.1 were
180 normalized in order to better highlight to what extent the peak broadening can decrease the
181 peak intensity and hence increase the signal-to-noise ratio (S/N). It is clear that the peak
182 shapes are significantly different depending on the nebulizer. Some peaks are symmetrical
183 but large, some others are thinner but with severe peak tailing. Very bad peak shapes always
184 result in low signal-to-noise ratios (S/N), well correlated with high peak variances. The worst
185 peak shape was obtained with the Burgener nebulizer which led to a measured peak
186 variance close to $1600 \mu\text{L}^2$. Such a significant dispersion can arise from the large internal
187 diameter (i.e. $380 \mu\text{m}$) of the connecting capillary located between UV-detection and the
188 nebulizer but also from the nebulizer itself due to its large orifice and its large internal
189 volume ($108 \mu\text{L}$). This latter has been designed to avoid the risk of clogging which makes this
190 nebulizer very attractive when particles or salts are present in the sample. Burgener is
191 expected to be ideally operated at high flow-rates (i.e. $> 800 \mu\text{L}/\text{min}$) rather than $400 \mu\text{L}/\text{min}$
192 as in the present study. This may contribute to further explain the very large peak observed
193 in Fig 1. Both Opalmist and Savillex nebulizers led to low S/N values in addition to significant
194 band broadening (peak variance of 653 and $136 \mu\text{L}^2$ respectively) making them also
195 inappropriate for UHPLC separations. The best peak shapes and hence the best S/N values
196 were obtained with PFA-LC or MicroMist as nebulizers. It can be noticed that both PFA-LC
197 and MicroMist were designed with low internal volumes, (i.e. 2 and $6 \mu\text{L}$ respectively),
198 thereby explaining the low peak variances. However, the variance value with MicroMist
199 ($92 \mu\text{L}^2$) was significantly higher than with PFA-LC ($6 \mu\text{L}^2$) which is due to their difference in
200 volume but also to their different design which can affect solute dispersion as highlighted by
201 the severe peak tailing in case of MicroMist (Fig.1). A comparison of the peaks obtained with
202 MicroMist (Fig.2a) or PFA-LC (Fig.2b) and different spray chamber is given in Fig.2. A
203 significant peak tailing along with low S/N can be observed with all studied spray chambers
204 when using MicroMist as nebulizer (Fig.2a). In contrast, the obtained peak shapes are much
205 better when using PFA-LC (Fig.2b) which also results in a higher S/N. One exception can be
206 noted in the case of Spectro-large providing a better peak shape with MicroMist (Fig.2a)
207 than with PFA-LC (Fig.2b). The advantage of PFA-LC over MicroMist can also be related to
208 the material which is made of perfluoroalkoxy (PFA) in case of PFA-LC while of borosilicate in
209 case of MicroMist. As a result, the observed peak tailing with MicroMist (Fig.2a) could be

210 explained by a higher wettability of borosilicate compared to PFA (contact angle of 38.5°
211 with water for borosilicate vs 98.5° for PFA) [11]. These nebulizers also differ by the recess of
212 the internal capillary. With PFA-LC, the capillary is more recessed than with MicroMist. As a
213 result, the two streams (gas and liquid) interact, thus promoting the formation of a fine
214 aerosol [12]. This latter might reduce solute dispersion, hence leading to a very low
215 dispersion variance when combining PFA-LC and Twinnabar (14 μL^2). Nevertheless,
216 calculated peak variances vary from 92 to 873 μL^2 with MicroMist while from 14 to 151 μL^2
217 with PFA-LC making this latter nebulizer of major interest for speciation analysis under
218 UHPLC conditions. Finally, it is important to note that for both nebulizers (PFA-LC and
219 MicroMist), S/N strongly depends on the spray chamber, making the selection of this latter
220 critical in many ways. The nine commercially available spray chambers, evaluated in this
221 study, also differed by their design and their volume. It is likely that some mixing occurred
222 into SIS. Depending on its design, the interface can be considered either as a tube or as a
223 mixing chamber. As shown in Fig.3, the variation of the peak variance with the volumes of
224 the different spray chambers seems to be well fitted by a parabolic curve, suggesting, for
225 most studied spray chambers, that a dispersion process took place, similar to that occurring
226 into a mixing chamber [13]. However, Agilent 8800, Spectro Small and Single-pass have
227 similar volumes (i.e. 70; 75 and 75 mL respectively) while quite different measured peak
228 variances (from 109 μL^2 to 370 μL^2). This suggests that the volume of the spray chamber is
229 not the only factor that can affect extra-column band broadening. Single-pass aerosol path is
230 likely to be different from Spectro Small and Agilent 8800 ones, both spray chambers being
231 double pass as detailed in Table 3. For the largest spray chamber (i.e. Spectro large; 150 mL),
232 the peak variance attained 143 μL^2 with PFA-LC while 865 μL^2 with MicroMist. These
233 differences can be ascribed to the design of the nebulizer itself as previously discussed. With
234 large spray chambers (above 40 mL), the total extra column variance was higher than 50 μL^2
235 with PFA-LC and higher than 90 μL^2 with MicroMist.

236 The loss in plates due to the instrument is expected to be directly related to the column
237 variance, σ_c^2 . As a result, it depends on both the retention factor, k and the column
238 geometry (column length, L_c , column internal diameter, d_c , and particle size, d_p). Based on
239 known relationships (Eq.2 to 4), it is possible to plot the variation of the remaining plates, β^2
240 (%) as a function of the retention factor, considering either HPLC or UHPLC conditions. The

241 corresponding curves are shown in Fig.4a and Fig.4b respectively, for different SIS (#3, #1,
242 #24, #2, #7, #4, #31, #21). β^2 is the ratio of the column variance, to the measured total peak
243 variance ($\beta^2 = \sigma_c^2 / \sigma_{total}^2$). It was calculated by using the following relationships:

$$244 \quad \sigma_c^2 = \frac{V_0^2 \times (1+k)^2}{L_c} \times h d_p \quad (2)$$

245 Where k is the retention factor, h , the reduced height equivalent to a theoretical plat, d_p ,
246 the particle size and V_0 the column dead volume given by

$$247 \quad V_0 = \frac{\pi \varepsilon_t d_c^2 \times L_c}{4} \quad (3)$$

248 Where ε_t is the total column porosity and d_c , the column diameter

$$249 \quad \sigma_{total}^2 = \sigma_{ext,total}^2 + \sigma_c^2 \quad (4)$$

250 leading to

$$251 \quad \beta^2 = \frac{1}{1 + \sigma_{ext,total}^2 / \sigma_c^2} \quad (5)$$

252 Calculations were performed by considering no other source of dispersion in the
253 instrument, $h=3$ and $\varepsilon_t=0.6$. For sake of clarity, only 7 curves, corresponding to 7 different SIS
254 interfaces (among 31 studied), were represented in Fig.4. SIS #1, #3, #2 and #24 are the most
255 widely reported in the literature. SIS #7 is interesting because it is the configuration
256 proposed by Agilent Technologies for the ICP-MS/MS instrument used in this study.
257 Although hardly reported, it is worth citing SIS #4, #31 and #21 because they led to very high
258 peak variances. In case of HPLC conditions (i.e. a 150 x 4.6 mm column packed with 5 μm
259 particles), maintaining more than 80% of plates with a retention factor lower than 2 appears
260 to be possible with four SIS only among the seven ones represented in Fig.4a. SIS #4 is
261 acceptable for retention factors higher than 4, SIS #31 for retention factors higher than 6
262 and SIS #21 for retention factors higher than 10. More generally, all SIS generating a peak
263 variance higher than 2000 μL^2 (see Table 4) are expected to be inappropriate for HPLC unless
264 accepting a dramatic loss in column plates. In case of UHPLC conditions (i.e. a 50 x 2.1 mm
265 column packed with sub-2 μm particles), this issue is much more critical considering the low
266 column volume involved. As shown in Fig.4b, no SIS allows to keep more than 80% of the
267 column plates with a retention factor of 2. For only two combinations of nebulizer and spray

268 chamber, namely SIS #3 (PFA-LC and Twinnabar) and SIS #1 (PFA-LC and IsoMist), the
269 percentage of remaining plates attains 80% provided that the retention factor is higher than
270 5. For the same retention factor of 5, the percentage of remaining plates significantly
271 decreases for both SIS #24 (PFA-LC and PC3) and SIS #2 (MicroMist and Twinnabar) (down to
272 50% and 30% respectively). Finally, all other SIS are expected to be quite inappropriate in
273 UHPLC unless all retention factors are kept far above 10 which does not fulfill the quality
274 standard in chromatography.

275 In light of the above, the top 4 ranked SIS for UHPLC conditions (Fig.4b) were found to be
276 SIS #3, #1, #24 and #2. They all led to extra-column peak variances lower than $85 \mu\text{L}^2$, far
277 below the next ranked SIS (i.e. SIS #7 with $370 \mu\text{L}^2$). These 4 configurations also correspond
278 to the most widely used ones compared to other ones reported in Table 4 (SIS #23, #12, #20
279 and #29) which could also be considered as attractive because they lead to similar peak
280 variances.

281 For the best three SIS (#3, #1 and #24), the nebulizer (i.e. PFA-LC) was the same. In order to
282 assess the reproducibility of our measures, these latter were performed over three
283 consecutive days. Considering the peak variance, the day-to-day variability was found to be
284 similar for the three configurations (RSD of about 30%), slightly higher than the run-to-run
285 variability reported in Table 4. Considering peak areas, the run-to-run variability was higher
286 with all SIS (RSD of 5 to 15%) than with UV detection alone (RSD of 1 to 2%). The severe
287 uncertainty on peak areas is strongly related to the specificity of the SIS interface and fully
288 explains the need for an internal standard in quantitative analysis in LC-ICP-MS/MS. The day-
289 to-day variability on peak areas was in the same order of magnitude except in case of SIS #3
290 (RSD of 50%). Such high variability can be related to the position of the PFA-LC tip which is
291 likely to be a key factor for achieving reproducible results. As highlighted by Todoli and
292 Mermet [12], the produced aerosol is not fully symmetrical. The position of the nebulizer
293 impacts on the signal, due to the recession of the capillary tip. The poor reproducibility of
294 peak area measurements can also be explained by the fact that the instrument setting was
295 in-house made whereas the Twinnabar spray chamber is not designed to be mounted alone
296 on the ICP-MS/MS instrument. No fittings were found to be optimal to properly fix the spray
297 chamber to the HMI tubing, resulting in a signal significantly affected by the variability of the
298 spray chamber position. The combined use of both IsoMist and temperature controlled

299 Twinnabar allowed us to address this latter issue. This new Twinnabar device (called IsoMist)
300 was indeed designed for ICP-MS/MS 8800. The resulting day-to-day variability on peak areas
301 was lower with this optimized configuration (SIS #1, RSD of 5%).

302 The preceding results were obtained without column, in flow injection analysis (FIA). They
303 allowed us to evaluate the impact of different commercial SIS on total extra-column
304 dispersion. From these results, SIS #1, #2 and #3 were found to be the most suitable for
305 future UHPLC-ICP-MS/MS analysis, considering both the extra-column dispersion and the
306 reliability in quantitative analysis. In order to emphasize these results, Vanadium 67 in a
307 porphyrin sample was analyzed by RPLC-ICP-MS/MS in UHPLC conditions using SIS #1, #2 and
308 #3 as interface between UV detection and ICP-MS/MS. Porphyrin peak obtained with UV
309 detection and ^{67}V peaks obtained with ICP-MS/MS detection using the 3 different interfaces
310 are shown in Fig.5. With acetonitrile as mobile phase and an Acquity BEH C18 (50 x 2.1 mm;
311 1.7 μm) column, the retention factor was close to 5. The peak variance was assessed from
312 the measured peak width at 10% of peak height using the Dorsey-Foley equation [14]. The
313 resulting measured total peak variance, σ_{total}^2 , allowed to determine the percentage of
314 remaining plates (i.e. $100 \times \sigma_c^2 / \sigma_{total}^2$, with σ_c^2 calculated according to Eq.2). The obtained
315 values are given above the peak apex in Fig.5. As expected the percentage of remaining
316 plates is very high with UV detection (88%) due to low extra-column volumes in UHPLC
317 system (low $\sigma_{ext,UHPLC/UV}^2$ values). SIS #1 and SIS #3 also allowed to keep a high percentage
318 of remaining plates (i.e. >80%) in good agreement with the curves shown in Fig.4, the larger
319 part of σ_{total}^2 , coming from σ_c^2 (k=5). With the last combination (SIS #2), the percentage of
320 remaining plates was below 30% (i.e. 28%), also is in good agreement with the theoretical
321 calculations shown in Fig.4 where the theoretical remaining plates with a UHPLC column was
322 found to be close to 30% for a retention factor of 5. It is important to underline that
323 although SIS #1 and SIS #3 led to such high percentage of remaining plates, that was
324 achieved for a rather high retention factor (k = 5) which means that for any less retained
325 peaks, the percentage of remaining plates should be below 80%. Anyway, under UHPLC
326 conditions, the combined use of PFA-LC and IsoMist (SIS #1) or alternatively, PFA-LC and
327 Twinnabar (SIS #3), was experimentally proved to be the best option.

328 **3.2. Effect of the spray chamber temperature**

329 Considering the spray chamber, an additional advantage of IsoMist (SIS #1) over Twinnabar
330 (SIS #3) is that its temperature can be modified. It was reported that the temperature into
331 the spray chamber may have an impact on the quality of the aerosol [15], suggesting an
332 impact on both dispersion and sensitivity. We therefore studied the effect of IsoMist
333 temperature (from -10°C to 8°C) on both peak variance and peak area. As shown in Fig.6, the
334 peak variance due to SIS seems to be not significantly affected by the temperature within
335 the studied range. In contrast, the peak area, and hence the detector sensitivity, is constant
336 up to -2°C while significantly decreases beyond. The impact of the spray chamber
337 temperature on detector sensitivity was often reported [8,16,17]. It was explained by the
338 fact that decreasing the solvent temperature reduces the solvent vapor pressure [8], thereby
339 reducing the amount of solvent per time unit that enters the plasma [18] and finally
340 improving the signal intensity. In this study, plasma parameters were kept constant when
341 the spray chamber temperature was changed. It is likely that plasma parameters and IsoMist
342 temperature might be better suited if they were optimized together. Nevertheless, this
343 possibility of increasing the signal by tuning the temperature offers an additional advantage
344 to IsoMist over all other studied spray chambers (Spectro Large, Spectro Small, Twinnabar,
345 Impact bead, Total consumption).

346 **3.3. Effect of the mobile phase flow rate**

347 The appropriate flow-rate for a given separation is dependent on theoretical
348 considerations taking into account the column internal diameter, the particle size, and the
349 solute diffusivity [19]. However it is well-known that the appropriate flow-rate for a
350 detection purpose may be quite different. The aim of this section was to evaluate, without
351 column, the impact of the flow rate (from 200 to 600 $\mu\text{L}/\text{min}$) on both σ_{SIS}^2 and the peak
352 area, using the two best SIS interfaces according to the above discussion. The variation of
353 σ_{SIS}^2 with the mobile phase flow-rate is shown in Fig.7 for SIS #1 (blue square) and SIS #3 (red
354 triangle). For both SIS, σ_{SIS}^2 increase linearly with the flow-rate (Fig.7a) which is well
355 supported by theoretical equations expressing the dispersion in an ideal tube [13]. σ_{SIS}^2 was
356 doubled between 200 $\mu\text{L}/\text{min}$ (6 μL^2) and 600 $\mu\text{L}/\text{min}$ (12 μL^2) which can affect less retained
357 compounds as highlighted in Fig.4b. However such variance values remain close to those

358 resulting from UHPLC/UV alone. The variation of peak area with the reciprocal of flow-rate
359 (Fig.7b) is quite different depending on SIS. For SIS #1 (blue squares) the peak area increases
360 linearly with the reciprocal of flow-rate which is the usual trend of a concentration
361 dependent detector (the detector response varies with the detector sensitivity and the
362 analyte concentration at the detector inlet). Unlike for SIS #1, for SIS #3, the peak area is
363 kept constant with the flow-rate (red triangles), which is in agreement with mass-flow
364 dependent detector (the detector response varies with the detector sensitivity, the analyte
365 concentration and the flow-rate at the detector inlet). As previously discussed, Twinnabar
366 and IsoMist, have the same concentric design as well as the same volume. The only
367 difference between both spray chambers is that IsoMist was specifically designed for ICP-
368 MS/MS 8800. In light of this, the difference in detection behavior was quite surprising and
369 not explained yet. However it is likely that SIS #3 should be better used with high flow-rates
370 to enhance peak intensity.

371 This study has highlighted the importance of selecting an appropriate flow-rate capable
372 to offer a good trade-off between column efficiency, low dispersion in SIS and high detector
373 sensitivity.

374 **Conclusion**

375 This study highlights the difference between a large set of Sample Introduction Systems
376 (SIS) in term of peak broadening. 6 nebulizers and 9 spray chambers, were compared under
377 optimized plasma conditions, offering the possibility to compare 31 combinations. The
378 difference in peak variance depending on commercially available SIS was found to be quite
379 impressive (from 10 μL^2 to 8000 μL^2). The repeatability on peak variance measure was much
380 higher with UHPLC-UV than with UHPLC-UV-ICP-MS/MS whatever SIS, suggesting a more
381 uncertain dispersion process in SIS. As shown in part I of this study, extra-column dispersion
382 should always be compared to the column variance, in order to minimize the loss in
383 theoretical plates. Accordingly, the present study gives some recommendations for a proper
384 selection of both nebulizer and spray chamber with respect to column geometry. Most of
385 current commercial SIS that provide less than 2000 μL^2 as extra-column variance, can be
386 used in conventional HPLC without significant loss in theoretical plates (< 20 %).
387 Unfortunately, only PFA-LC in combination with IsoMist or Twinnabar as spray chamber (SIS

388 #1, and SIS #3 respectively in this study) can be considered as suitable for UHPLC
389 separations.

390 It has been shown that both nebulizer and spray chamber should be selected together.
391 MicroMist nebulizer leads to severe peak tailing which is likely due to its raw material
392 (borosilicate material) compared to PFA-LC. Whereas in most cases, PFA-LC provides the
393 lowest variance values, compared to other nebulizers, the spray chamber has also to be
394 chosen carefully to avoid additional peak variance. Reducing the volume of the spray
395 chamber can usually lower solute dispersion.

396 The design of SIS affects the peak shape but also may affect the sensitivity. The study of
397 the effect of the spray chamber temperature on both peak variance and peak intensity
398 showed that the first one was not significantly affected by a change in temperature while
399 the second one decreased drastically when temperature increased. These observations were
400 in good agreement with previous studies using organic solvents in the mobile phase. Finally,
401 the study of the impact of the mobile phase flow rate on both peak variance and peak area
402 highlighted a difference in ICP-MS/MS behavior, working either as a concentration
403 dependent detector or as a mass flow dependent detector depending on SIS.

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474

475 **Figure captions**

476
477 Figure 1 Peaks obtained without column using the Twinnabar spray chamber and five
478 different nebulizers: SIS #4 Burgener; SIS #5 Opalmist; SIS #6 Savillex; SIS #2 MicroMist; SIS
479 #3 PFA-LC; UHPLC/UV System. Sample: porphyrin (10 ppm in THF). Detected element: ⁶⁷V.
480 Total extra-column peak variances are given above the peak apex (in μL^2). 1 μL injected. No
481 column. Mobile phase: ACN. 407 nm. 400 $\mu\text{L}/\text{min}$.

482
483 Figure 2 Peaks obtained without column using two different nebulizers: (a) Micromist and 7
484 different spray chambers: SIS #15 Spectro Large; SIS #30 Spectro Small; SIS #11 Impact bead;
485 SIS #7 Agilent 8800; SIS #2 Twinnabar; SIS #19 Single pass; SIS #25 PC3; UHPLC/UV and (b)
486 PFA-LC and 9 different spray chambers: SIS #16 Spectro Large; SIS #29 Spectro Small; SIS #20
487 Single pass; SIS #3 Twinnabar; SIS #12 Impact bead; SIS #23 Total; SIS #8 Agilent 8800; SIS #1
488 IsoMist; SIS #24 PC3; UHPLC/UV. Total extra-column peak variance are given at peak apex (in
489 μL^2). Flow rate: 400 $\mu\text{L}/\text{min}$ for every Spray chamber except for Total spray chamber Flow
490 rate: 200 $\mu\text{L}/\text{min}$. Same other conditions as in Fig.1.

491
492 Figure 3 SIS peak variance as a function of the volume of the spray chamber with PFA-LC (■)
493 and MicroMist (▲) as nebulizers. Each data point corresponds to a specific spray chamber
494 among nine listed in Table 2

495
496 Figure 4 Percentage of remaining plates as a function of retention factor for different column
497 geometries (a) HPLC column (150 x 4.6 mm, 5 μm) and (b) UHPLC column
498 (50 x 2.1 mm, 1.7 μm) for different SIS with #3: $\sigma_{SIS}^2 = 6 \mu\text{L}^2$; #1: $\sigma_{SIS}^2 = 11 \mu\text{L}^2$; #24:
499 $\sigma_{SIS}^2 = 42 \mu\text{L}^2$; #2: $\sigma_{SIS}^2 = 84 \mu\text{L}^2$; #7: $\sigma_{SIS}^2 = 370 \mu\text{L}^2$; #4: $\sigma_{SIS}^2 = 1582 \mu\text{L}^2$; #31: $\sigma_{SIS}^2 =$
500 $3769 \mu\text{L}^2$; #21: $\sigma_{SIS}^2 = 8070 \mu\text{L}^2$. Calculations from Eq.2 to 5, with $\epsilon_t = 0.60$, $h = 3$. SIS
501 numbered in Table 4

502
503 Figure 5 Peaks obtained with ACQUITY BEH C18 column (50 x 2.1 mm, 1.7 μm) using
504 different Sample Introduction Systems and with UV detection: SIS #2 Micromist + Twinnabar;
505 SIS #3 PFA-LC + Twinnabar; SIS #1 PFA-LC + IsoMist; UHPLC/UV system; Fraction of remaining
506 plates, β^2 , are given above the peak apex (Calculations from Eq.2 to 5, with $\epsilon_t = 0.60$,
507 retention factor of 5, and $h=3$). Same conditions as in Fig.1.

508
509 Figure 6 Total peak variance (■) and peak area (▲) as a function of the spray chamber
510 temperature (IsoMist) with the PFA-LC nebulizer (SIS #1). Same conditions as in Fig.1.

511
512 Figure 7 (a) SIS peak variance and (b) peak area as a function of the mobile phase flow-rate
513 with two combinations of nebulizer and spray chamber: #1-PFA-LC + IsoMist (■), #3-PFA-LC
514 + Twinnabar (▲). Same conditions as in Fig. 1

515













