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

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Abstract

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

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

Keyword

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

1. Introduction

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

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

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

2. Experimental section

2.1 ICP-MS/MS instrument and conditions

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

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

2.2 UHPLC instrument conditions

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

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

2.3. Chemicals and sample preparation

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

3. Results and discussion

3.1. Comparison of 31 commercially available interfaces

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

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

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

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

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

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

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

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

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

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

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

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

$$\sigma_C^2 = \frac{V_0^2 \times (1+k)^2}{L_C} \times h d_p \quad (2)$$

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

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

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

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

leading to

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

Calculations were performed by considering no other source of dispersion in the instrument, $h=3$ and $\varepsilon_t=0.6$. For sake of clarity, only 7 curves, corresponding to 7 different SIS interfaces (among 31 studied), were represented in Fig.4. SIS #1, #3, #2 and #24 are the most widely reported in the literature. SIS #7 is interesting because it is the configuration proposed by Agilent Technologies for the ICP-MS/MS instrument used in this study. Although hardly reported, it is worth citing SIS #4, #31 and #21 because they led to very high peak variances. In case of HPLC conditions (i.e. a 150 x 4.6 mm column packed with 5 μ m particles), maintaining more than 80% of plates with a retention factor lower than 2 appears to be possible with four SIS only among the seven ones represented in Fig.4a. SIS #4 is acceptable for retention factors higher than 4, SIS #31 for retention factors higher than 6 and SIS #21 for retention factors higher than 10. More generally, all SIS generating a peak variance higher than 2000 μ L² (see Table 4) are expected to be inappropriate for HPLC unless accepting a dramatic loss in column plates. In case of UHPLC conditions (i.e. a 50 x 2.1 mm column packed with sub-2 μ m particles), this issue is much more critical considering the low column volume involved. As shown in Fig.4b, no SIS allows to keep more than 80% of the column plates with a retention factor of 2. For only two combinations of nebulizer and spray

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

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

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

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

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

3.2. Effect of the spray chamber temperature

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

3.3. Effect of the mobile phase flow rate

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

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

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

Conclusion

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

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

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

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

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Figure captions

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

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

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

Figure 4 Percentage of remaining plates as a function of retention factor for different column geometries (a) HPLC column (150 x 4.6 mm, 5 μm) and (b) UHPLC column (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: $\sigma_{SIS}^2 = 42 \mu\text{L}^2$; #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: $\sigma_{SIS}^2 = 3769 \mu\text{L}^2$; #21: $\sigma_{SIS}^2 = 8070 \mu\text{L}^2$. Calculations from Eq.2 to 5, with $\epsilon_t = 0.60$, $h = 3$. SIS numbered in Table 4

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

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

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













