Suitable interface for coupling Liquid Chromatography to Inductively Coupled Plasma-Mass Spectrometry for the analysis of organic matrices. 1 theoretical and experimental considerations on solute dispersion

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Abstract

Liquid chromatography (LC) hyphenated to a specific detection such as inductively coupled plasma-mass spectrometry (ICP-MS) is a technique of choice for elemental speciation analysis. However, various instrumental limitations may considerably reduce the expected sensitivity of the technique. Among those, we were interested by the solute dispersion into the interface located between LC and ICP-MS. The interface consists of a Sample Introduction System (SIS) and a possible flow-splitter prior to SIS. Flow splitting can be required in case of organic matrices to reduce the organic solvent amount entering plasma which may lead to plasma instabilities.

Although extra-column dispersion is usually well taken into account with conventional UV detection it has been little studied in the context of LC-ICP-MS and moreover never quantified. Our objective is to assess the loss in column plates and hence in both separation quality and sensitivity which may be generated by the coupling of LC and ICP-MS in the specific case of organic matrices. In this first study, this is done (1) from a theoretical approach; (2) from 55 experimental studies reported in LC-ICP-MS and (3) from our experimental results highlighting the critical impact of the flow splitter on extra-column dispersion depending on both flow-rate and split ratio. It turns out by evaluating the 55 reported studies by means of theoretical calculations, that the loss in plates due to extra-

column dispersion was most of the time beyond 50 % and even often beyond 90 %. Moreover, from our experiments, it has been shown that a very low split ratio (1:50) could generate an additional variance around 200 μL^2 which induces a loss in theoretical plate of 90 % for ultra-high performance LC (UHPLC) column (5 cm x 2.1 mm, 1.7 μm).

Keywords

Speciation - Liquid chromatography - Inductively coupled plasma - Extra-column dispersion - Sample introduction system - LC-ICP-MS – Flow splitting

1. Introduction

The need for determining elemental species concentrations rather than total element concentrations has grown within the past decades. According to Templeton et al. [1,2] speciation analysis consists in identifying and quantifying different chemical species of a particular element in a given sample. It has become an important field of research over the past few years in several areas including biochemistry, environmental chemistry, ecotoxicology, pharmaceuticals, petrochemicals, and nutrition science [3].

Nowadays hyphenated techniques such as Liquid Chromatography (LC) hyphenated with Inductively Coupled Plasma Mass Spectrometry (LC-ICP-MS) are widely used both to obtain elemental information and to discriminate species in a given matrix. LC techniques, including ion exchange chromatography (IEC) [4], reversed-phase liquid chromatography (RPLC) [5], ion-pairing chromatography (IPC) [6], size exclusion chromatography (SEC) [7,8] and hydrophilic interaction liquid chromatography (HILIC) [9,10], have been used for speciation analysis [11]. Different hyphenated techniques can be combined together to achieve more exhaustive characterization of complex matrices. For example, LC-ESI-MS can be associated to LC-ICP-MS to obtain both structural information and elemental information [5,12–14].

Most speciation analyses are performed in aqueous matrices by using ion-exchange chromatography and deal with environmental samples [15]. In this specific case, the mobile phase is not critical for the coupling of both techniques since the amount of organic solvent in the mobile phase is limited (small percentage of organic modifier sometimes added, usually without disturbing plasma stability). However, when using RPLC [16–28] or HILIC [9,10,29–34], a large amount of organic solvent is introduced into the plasma. The problems

involved by this introduction was thoroughly discussed by Leclercq et al [35,36] in a recent review.

Some key issues have to be considered for coupling LC to ICP-MS in case of organic matrices [3]: (i) metal contamination from the chromatographic system and/or the stationary phase and/or the mobile phase [3], (ii) plasma instabilities due to the solvent load, especially in case of organic mobile phases [3], (iii) signal fluctuations in gradient elution depending on plasma parameters [37] and (iv) solute dispersion into the interface located between LC and ICP-MS. The interface 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 solvent entering plasma and hence to decrease plasma instabilities [3]. The solute dispersion in the interface unit is a critical issue because that can result in additional solute band broadening and hence in significant loss in both sensitivity and separation quality. Although extra-column dispersion is usually well taken into account with conventional UV detection, it has been little studied in the context of LC-ICP-MS. In the present study, we made therefore an attempt to assess the extent to which the interface contributes to solute band broadening. This was done by (i) estimating from published studies the likely loss in plates due to solute dispersion in the interface and (ii) showing the critical impact of the flow splitter on extra-column dispersion depending on both flow-rate and split ratio. To support the first approach, a synoptic table has been built (Table 2) which summarizes 55 studies carried out on organic matrices in LC-ICP-MS and gives, for each study, an estimation of the interface contribution to solute band broadening. A further second part of our study will be dedicated to the comparison of a large number of commercially available SIS regarding the extra-column dispersion.

2. Theoretical considerations

Solute dispersion can be assessed by the peak variance. The total solute dispersion (total variance, σ_{total}^2) comes from both dispersion inside the column (column variance, σ_{col}^2) and extra-column dispersion (extra-column variance, σ_{ext}^2).

Extra-column dispersion results from the injection process ($\sigma_{injection}^2$), the different tubing (σ_{tubing}^2) and the detection ($\sigma_{detector}^2$) [38].

87 Because variances can be added if the corresponding dispersion process are independent of
 88 each other, the total peak variance can be written as

$$89 \quad \sigma_{total}^2 = \sigma_{col}^2 + \sigma_{ext}^2 \quad (1)$$

90 Similarly, the extra-column variance is the sum of individual contributions according to

$$91 \quad \sigma_{ext}^2 = \sigma_{injection}^2 + \sigma_{tubing}^2 + \sigma_{detector}^2 \quad (2)$$

92 For Gaussian peaks, the total peak variance in volume units can be given by the measured
 93 peak width at half peak height ($w_{0.5}$) according to

$$94 \quad \sigma_{total}^2 = \frac{F^2 w_{0.5}^2}{5.54} \quad (3)$$

95 Where F is the mobile phase flow-rate.

96 For very bad peak shapes, the second order central moment has to be used to provide a
 97 reliable variance value. These latter, in volume units, is given by

$$98 \quad \sigma_{total,v}^2 = F^2 \frac{\int_0^\infty (t - t_R)^2 I(t) dt}{\int_0^\infty I(t) dt} \quad (4)$$

99 Where t_R is the mean residence time and $I(t)$ the intensity as a function of time.

100 Extra-column variance can be approximated by removing the column and replacing it by a
 101 zero dead volume union connector. In this case, extra-column variance is calculated from
 102 Eq. (3).

103 The column variance, expressed in length unit is related to both the column length and the
 104 column plate height, H_{col} , by

$$105 \quad \sigma_{col}^2 = L H_{col} \quad (5)$$

106 H_{col} varies with the mobile phase linear velocity, u , and its variation may be fitted by the
 107 van Deemter equation [39] or by the Knox equation [40] using reduced parameters h_{col}
 108 (H_{col}/d_p) and v ($u \cdot d_p / D_m$, d_p being the average particle diameter and D_m , the molecular
 109 diffusion coefficient of the solute in the mobile phase). At the minimum of the curve, typical
 110 values for h_{col} and v are 3 and 5 respectively.

111 Considering the solute linear velocity at the time the solute is eluted from the column
 112 ($u/(1+k_e)$, k_e being the retention factor at elution), the column variance can be expressed in
 113 volume units according to

$$114 \quad \sigma_{col}^2 = \frac{V_0^2(1+k_e)^2 H_{col}}{L} \quad (6)$$

115 V_0 is the column dead volume, related to the column length, the internal diameter, d_i and
 116 the column porosity, ε_t by

$$117 \quad V_0 = \pi L \varepsilon_t (d_i^2/4) \quad (7)$$

118 Under isocratic conditions, k_e depends on the retention volume and is given by

$$119 \quad k_e = \frac{V_R}{V_0} - 1 \quad (8)$$

120 Under gradient conditions, k_e depends on both the gradient conditions and the solute
 121 properties. However a rough estimation of k_e can be made when the linear solvent strength
 122 theory (LSST) can be applied and the solvent strength parameter, S is known [41,42], using
 123 the following relation

$$124 \quad k_e = \frac{1}{2.3 S t_0 \frac{\Delta C}{t_G}} \quad (9)$$

125 With t_0 the column dead time, ΔC the gradient composition range, and t_G the gradient time.
 126 Typical values of S (S being the absolute value of the slope of of the linear relationship
 127 between the logarithm of the retention factor and the stronger solvent volume fraction) are
 128 4 for small molecules, 20 for peptides and much higher for larger molecules [42].

129 Thus, From Eqs. (6) and (7), the column variance for a given peak in a given chromatogram
 130 can be estimated provided that the column geometry is known, and the retention factor can
 131 be determined.

132 Similarly to Eq. (6), the total variance can be written

$$133 \quad \sigma_{total}^2 = \frac{V_0^2(1+k_e)^2 H_{total}}{L} \quad (10)$$

134 Where H_{total} is the total plate height resulting from the two dispersion processes.

135 The ratio, β^2 , between column and total variances corresponds to the ratio between the two
 136 plate heights and hence to the ratio between effective and column plate numbers according
 137 to

$$138 \quad \beta^2 = \frac{\sigma_{col}^2}{\sigma_{total}^2} = \frac{H_{col}}{H_{total}} = \frac{N_{effective}}{N_{col}} \quad (11)$$

139 Where $N_{effective}$ and N_{col} are effective and column plate numbers respectively.

140 The term, β^2 represents the fraction of remaining plates for a given solute, in given
 141 chromatographic and instrumental conditions.

142 The peak height and the resolution between two peaks are inversely proportional to the
 143 peak standard deviation (σ). As a result, the fractions of remaining peak height and
 144 remaining resolution are given by β .

145 The mobile phase flow-rate is related to the reduced linear velocity by

$$146 \quad F = v \frac{V_0}{L} \frac{D_m}{d_p} \quad (12)$$

147 From Eqs. (6) and (12), Table 1 gives an overview of optimum flow-rates and corresponding
 148 column variances ($k_e=3$) depending on both column internal diameter and particle size.

149 Depending on SIS, the flow entering the plasma source is expected to be a critical parameter
 150 regarding the performance of ICP-MS. According to Table 1, the internal diameter has to be
 151 chosen in accordance with the required flow-rate. However as shown in Fig.1, any decrease
 152 in column internal diameter leads to a severe decrease in column variance, and hence to a
 153 huge decrease in both plate number and sensitivity if the dispersion in SIS is significant. The
 154 percentage of remaining plates was calculated from Eq. (11) as a function of extra-column
 155 variance for different column internal diameters and two different particle sizes (5 and
 156 1.7 μm). The calculations were performed for a k_e value of 3 and a column length providing
 157 10000 plates (Fig.1a. 15 cm with 5 μm and 5 cm with 1.7 μm) and 30000 plates (Fig.1b 15 cm
 158 with 1.7 μm). Extra-column variance was considered in the range 0.1 to 1000 μL^2 that covers
 159 current LC-UV instruments except nanoLC instruments. With the aim of maintaining more
 160 than 80 % of the plates ($\beta^2 > 0.8$) and hence more than 90 % of the peak height ($\beta > 0.9$), it
 161 appears that the maximum allowable values for the extra-column variance are (1) 1000 and
 162 100 μL^2 for conventional columns (4.6 mm i.d) packed with 5 μm and 1.7 μm particles

respectively; (2) 50 and 5 μL^2 for narrow bore columns (2.1 mm i.d) packed with 5 μm and 1.7 μm particles respectively; 3 and 0.3 μL^2 for micro bore columns (1 mm i.d) packed with 5 μm and 1.7 μm particles respectively. These values are slightly higher for 30000 plates with 1.7 μm particles (Fig.1b). With capillary columns (300 μm i.d.), the extra-column variance should be much lower than 0.1 μL^2 . This figure also highlights the range of extra-column variance covered by commercially available HPLC and UHPLC instruments with UV detection. With the objective of reaching 10000 plates, it appears that HPLC-UV instruments are not suitable for conventional columns packed with 1.7 μm particles and that most UHPLC-UV instruments cannot be used with narrow bore columns packed with 1.7 μm particles. Furthermore, even very efficient UHPLC-UV instrument (i.e. extra-column variance as low as 5 μL^2) are not suitable for micro bore columns.

In this context, it is of prime importance to assess the additional extra-column variance brought by the interface between LC and ICP-MS. Considering the curve shapes in Fig.1, it appears that, in any case, the remaining plates might decrease from 80 % to 70 %, 50 %, and 30 % by increasing the extra-column variance by a factor of 2, 5 and 10 respectively.

As previously discussed, the interest for LC-ICP-MS in case of organic matrices has significantly grown during the past few years. In our opinion, instrument performances are often not considered enough, methods being developed without prior evaluation of the interface contribution to solute dispersion. To assess the additional dispersion brought by the interface in LC-ICP-MS, 55 studies reported in the area of organic matrix speciation have been reviewed and discussed in term of extra-column dispersion in the next section. With a view to reducing the flow entering SIS, an alternative to the reduction of internal diameter may be the use of a flow-splitter prior to SIS. A T-union was indeed used for quantification with online isotopic dilution [25,43–48]. However such unit may lead to significant additional extra-column band broadening. The contribution of flow splitting to extra-column dispersion is therefore studied in the last section.

3. LC-ICP-MS Interface

Peak band broadening is rarely taken into consideration even when the sample matrix becomes complex. Most of the time, authors work with only a few standards instead of a complex mixture to optimize the separation techniques and even less often on a real

sample. For instance, Raber et al. [49] focused on eleven arsenic species using an LC separation prior to ICP-MS detection but, as can be seen, the peaks were poorly resolved. A low measured plate number with respect to the theoretical column plate number, can be due to (i) solute dispersion in the chromatographic system (injection system, tubing, UV-detection), (ii) a loss in column plates (depending on the history of the column), or (iii) an additional dispersion generated by the interface between LC and ICP-MS. The interface consists in a Sample Introduction System (SIS) and a possible flow-splitter prior to SIS which can be required, in case of organic matrices, to reduce the amount of solvent entering plasma and hence to decrease plasma instabilities. SIS is usually divided into two devices, the nebulizer, and the spray chamber. The nebulizer converts the liquid from the separation technique into a heterogeneous aerosol made of different droplet sizes. The aerosol is then usually sorted out in a spray chamber. Larger droplets are carried to the waste while smaller ones are sent to the plasma source for atomization/excitation/ionization [35]. Nebulizers, spray chambers, and flow-splitters represent critical devices for ensuring a high-performance coupling. Particular attention must be paid both to the quality of the aerosol produced through the nebulizer/spray chamber [50] and to the contribution of the whole interface to peak band broadening [3,44,45,51–53], both features being able to significantly affect the sensitivity. A well-documented summary of SIS devices was reported by Leclercq and al. [36]. Major advantages and drawbacks were discussed in terms of sensitivity and ease of use, but other analytical performances such as efficiency, resolution, sensitivity or extra-column solute dispersion, were not taken into account to compare the different devices. In the present work, this feature has been subjected to a broad exploratory study on 55 published studies (from 1995 to 2017) dealing with the speciation of organic matrices. The corresponding analytical conditions, including LC conditions, type of nebulizers, type of spray chambers and use or not of a flow-splitter, are listed in Table 2 and Table S1 of Supplementary Information, depending on whether enough data were available or not. When data were available (Table 2), an evaluation of the total solute dispersion (total variance, σ_{total}^2) was made from Eq. (3) by considering the most retained peak (symmetrical peaks only). The column variance, σ_{col}^2 , was evaluated from Eq. (6). The extra-column variance, σ_{ext}^2 , was estimated from Eq. (1). To easily compare the percentage of remaining plates (β^2), this value was systematically determined for a retention factor of 3, by column

variance rescaling using Eq. (6). Corresponding β^2 values are given in Table 2. The reasons for low β^2 values can be strongly related to the instrument characteristics including the type of interface but also the level of matching between the column geometry and the LC instrument.

Fig.2 illustrates the relative distribution of nebulizers and spray chambers used for speciation in LC-ICP-MS in case of organic solvent matrices regarding the dimension of the column used. In many studies, it was difficult to draw relevant conclusions because of a lack of information about SIS and/or the column particle diameter and/or even sometimes, the mobile phase flow-rate. However when the calculations were possible, the following comments can be provided depending on whether the column was conventional, narrow-bore, micro-bore or capillary:

(i) conventional columns:

According to Fig.2, three different nebulizers have been used: pneumatic micro-concentric nebulizer (50 %) operated with flow-rates in the range 40 to 1000 $\mu\text{L}/\text{min}$, pneumatic concentric nebulizer (10 %) operated with flow-rates higher than 1000 $\mu\text{L}/\text{min}$ and hydraulic high-pressure nebulizer (10 %). With the first and second ones [17,20,22,24,25], the liquid is introduced through a horizontal capillary and the gas conducted through an external tube around the liquid capillary. With the third one [54], the liquid is forced through a highly turbulent hydraulic nozzle. Similarly, three different spray chambers are used in association with the preceding nebulizers: cyclonic spray chambers (30 %), double pass spray chamber (20 %) and desolvation units (10 %). With cyclonic spray chambers, the aerosol is tangentially introduced and first impacts against the front walls generate thinner aerosol which is transported towards the injector [50]. The double pass spray chambers is composed of two concentric tubes, the external one promoting droplet elimination through impacts against the wall and reducing aerosol fluctuation due to pump pulsing, the internal one eliminating coarse droplets and inducing a laminar flow aerosol [50]. Finally, desolvation units are designed to reduce the solvent amount into the plasma with either a desolvation membrane to remove organic/hydro-organic matrices in association with a micro-concentric nebulizer [16,54,55] or an ultrasonic nebulizer [56]. The main drawback of desolvation systems is the possible loss of analytes through the desolvation membrane. As reported, the sensitivity

achieved with a desolvation micro-concentric nebulizer was dependent on the analyte structure [55,57].

When calculations were possible, the calculated percentage of remaining plates (β^2) was found to be lower than 35 % (i.e. 24 [17], 16 % [24], 31 % [20], 14 % [25], 34 % [58]) except in one case (i.e. 86 % [26]). Unfortunately, in this latter case, the information about the interface was not available and as a result, no relevant conclusion could be drawn. As seen in Fig.2 and mentioned earlier, in many cases, the necessary information about nebulizer (30 %) and spray chamber (40 %) was not provided. It is important to note that, with conventional columns leading to high column variance values, the measured loss in plates is likely due either to the interface or to column ageing or to both, but probably not to the contribution of LC instrument to extra-column solute dispersion. As a result, it can be concluded that, in most cases, the separation achieved in the column was partially or completely lost in the interface. As examples, with a micro-concentric nebulizer in association with a Peltier Cooled Cyclonic spray chamber PC³ (supplied by Elemental Scientific), only 24 % of the plates remained [17] and 14 % in association with a double pass spray chamber [25]. It can be noticed that only one study deals with the use of low cyclonic spray chamber among the 55 publications reported [52]. Finally, in association with a desolvation unit, the very bad peak shapes did not allow to conclude about the impact of the interface [54].

(ii) Narrow bore columns

The distribution of the nebulizers was different from that for conventional columns with four different ones used: micro-concentric nebulizers (67 %), parallel path nebulizers (12 %) with the liquid interacting with the high-velocity gas stream coming tangentially into contact with it [50], cross-flow nebulizers (4 %) with liquid and gas outlets perpendicularly mounted on a polymer body and concentric nebulizers (4 %). Most of the time, micro-concentric nebulizers [9,19,21,23,27–29,33,34,47,52,54,59–64] were associated to a cyclonic spray chamber (54 % of the spray chambers used). A specific micro-concentric nebulizer (i.e. microflow PFA-ST or PFA-LC nebulizer supplied by Elemental scientific) was used in several studies [59,61,64]. It is made of a copolymer, with an internal capillary more recessed than other micro-concentric nebulizers [50]. However, very low percentages of remaining plates were estimated in these cases. The highest value was 70 % [52] while all other ones were below 20 % and even some

of them below 5 % [29,60]. In the particular study of Vidler et al. [59], the calculated retention factor was found to be lower than 1 (i.e. about 0.5) leading to 16 % of remaining plates only. In this study a micro concentric nebulizer was used, unfortunately, the spray chamber was not mentioned. However, considering a retention factor of 3, the calculated fraction of remaining plates is tremendously improved (i.e. 59 %), suggesting that, in this particular case, the loss in plates was likely due to LC system volumes rather to the interface. Interestingly, the use of two different detectors, ICP-MS and ESI-TOF [33] allowed us to indirectly assess the extra-column dispersion induced by the interface between LC and ICP-MS, assuming no dispersion in both ESI-TOF and ICP-MS. From our calculations, the total variance was 1360 μL^2 with LC-ICP-MS against 490 μL^2 with LC-ESI-MS, thereby leading to an impressive peak variance of 870 μL^2 for the interface alone, far too high considering the expecting narrow-bore column variance (i.e. <200 μL^2 as shown in Table 1). Unfortunately, no information about the interface was available [33] which could have permitted to draw some interesting trends.

(ii) Micro bore and capillary columns

When the column internal diameter is further decreased, the trend is towards the use of total consumption nebulizers (35 % and 18 % for microbore and capillary columns respectively) at the expense of micro-concentric ones (67 %, 47 % and 18 % for narrow-bore, microbore and capillary columns respectively) (Fig.2). Total consumption nebulizers do not require spray chambers or desolvation systems and can work with low flow-rates (typically a few tens of $\mu\text{L}/\text{min}$). The primary aerosol is entirely sent to the plasma. Two different total consumption nebulizers were used with organic/hydro-organic matrices, namely Direct Injection Nebulizer (DIN, supplied by CETAC Technologies) and Direct Injection High-Efficiency Nebulizer (DIHEN, supplied by Meinhard). DIN has been used since 1992 [65] and was often adapted before use [16, 65]. Despite its low volume, highly recommended for hyphenated techniques, its main drawback is its small internal capillary (i.e. 60 μm) which can be easily clogged [36,50]. Moreover, finding the correct distance between the tip and the plasma is not easy considering the capillary thinness. For the above reasons, this nebulizer is no more commercialized [66]. DIHEN is a pneumatic nebulizer in glass or quartz, working with liquid flow-rates between 1 and 100 $\mu\text{L}/\text{min}$. It is characterized by a larger dead volume than DIN. It is therefore always used with an inserted capillary to reduce its dead

volume [36,44,45,51,67–69]. The optimum flow-rate for total consumption nebulizers is a few $\mu\text{L}/\text{min}$, which is also the range of flow-rates adapted to capillary LC (i.e. $< 10 \mu\text{L}/\text{min}$ as seen in Table 1). Nevertheless in all studied cases (see Table 2), the estimated percentage of remaining plates was lower than 5 %.

Finally, two different SIS (a total consumption nebulizer and a micro-concentric nebulizer combined with a cyclonic spray chamber) were compared for a given capillary column while keeping the same separation conditions [67]. In both cases, the estimated percentage of remaining plates was found to be lower than 1 %, highlighting once again the importance of extra-column band broadening, especially in case of thin columns and the necessity of trying to ensure the lowest possible contribution of the interface.

In summary, it has been pointed out that it is essential that the interface is optimized in relation to the separation conditions and especially to the column geometry. In most cases among 26 published chromatograms, the estimated percentage of remaining plates (calculated with a retention factor of 3) were found to be lower than 50 %, 20 %, 5 % and 1 % for conventional, narrow-bore, micro-bore and capillary columns respectively. These low percentage values clearly show that the interfaces are mostly inappropriate. Moreover, it is important to point out that the extra column dispersion is never considered. Further investigations were carried out in our Lab to compare commercially available SIS with respect to extra-column dispersion. These results will be extensively discussed in Part II.

Adapting the flow-rate before entering SIS can be a good option when using a total consumption nebulizer designed to work at low flow-rates to limit the amount of organic solvent entering the plasma. A zero-dead volume T-union along with suitable tubing can be used to adjust the flow-rate just prior entering SIS [12,25,44,45,67]. Nonetheless, such device is also expected to give rise to additional extra-column dispersion. Zoorob et al. [45] indeed showed that a split ratio of 1:20 generated more dispersion in the interface than DIN alone. However, most authors, using a flow splitter did not consider the additional dispersion generated by such devices [24,25,44,67]. However, it was evaluated under fast separation conditions by injecting, without column, 20 μL of a potassium iodide solution [47]. Flow splitting was used to reduce the acetonitrile concentration and hence to improve ICP-MS performance. The authors visually compared peak shapes with and without flow splitting and deduced that the T-connection did not have a significant impact on the

dispersion. However, the comparison was qualitative and not quantitative. To the best of our knowledge, no quantitative study on additional variance brought by flow splitting depending on both flow-rate and split ratio has been reported. This issue is addressed in the next section.

4. Determination of peak variance resulting from flow splitting

The aim of the present study was to quantify the additional peak variance due to the presence of a flow-splitter. As discussed above, total consumption spray chambers can be used with low flow-rates (up to a few tens of $\mu\text{L}/\text{min}$) to reduce the solvent load into the plasma. However, such flow-rates are much too low when using narrow bore columns (i.e. 2.1 mm i.d.) and sub 2 μm particles as usually done in UHPLC. A flow-splitter can be used to solve this problem. It consists in a zero-dead volume T-union located between the column outlet and the detector and intended to divide the main flow into two different flows, the highest being directed to the waste and the lowest to the detector, thereby significantly reducing the flow entering the plasma source. The difficulty may stem from the volumes involved by additional tubing and from the split itself as both may lead to significant additional band broadening, especially when a low split ratio has to be considered. This issue is addressed below by assessing the extra-column variance induced by such device depending on flow-rate, internal diameter of tubing and split ratio.

4.1. Material and reagents

Methanol was HPLC grade from Sigma Aldrich (Steinheim, Germany). Water was obtained from Elga water purification system (Veolia water STI, Le Pless Robinson, France). Methylparaben was used as test compound and supplied by Sigma-Aldrich (Steinheim, Germany).

4.2. Apparatus

The instrument used was an Acquity UPLC I-Class 2DLC liquid chromatography system. It includes two high-pressure binary solvent manager with a maximum delivery of 2 mL/min, an autosampler with a 5 μL injection sample loop, a column oven with a maximum temperature of 90°C and two different detectors (TUV and PDA) with identical flow-cells,

leading to identical signal intensity. They can be used one after the other with or without flow-splitter between both. The wavelength was set at 254 nm with a sampling rate of 40 Hz. The instrument control was performed by Mass Lynx software. The maximum backpressure allowed in the first detector cell was 1000 psi.

4.3. Procedure and methods

The mobile phase was a mixture of 50/50 Water/MeOH (v/v). Methylparaben was diluted in the same mixture with a concentration of 75 ppm. This study was carried out without column. All peak variances were calculated from the second order moment of the peak, Eq. (4), using in-house calculation tool. The injected volume was 1 μ L. A P-727 (0.57 μ L) T-union was used for flow splitting (from Upchurch, Cluzeau, Sainte-Foy-La-Grande, France). This T-union was chosen according to a previous study on different commercially available T-unions which concluded that this one was the best adapted to low dispersion [70]. The set-up used to measure the peak variance due to flow splitting is shown in Fig.3. Tube #1 was located between UV-detector #1 and the T-union, Tube #2, between the T-union and UV Detector #2 (replacing ICP-MS detection for this study) and Tube #3 between the T-union and the waste. Four different settings were considered. The corresponding tubing geometries are given in Table 3. The theoretical split ratio, z_{th} , is calculated from the Poiseuille law, considering the dimensions of Tube #2 and #3, Eq. (13):

$$z_{th} = \frac{R_3}{R_3 + R_2} \text{ with } R_i = \frac{L_i}{d_i^4} \quad (13)$$

L_i and d_i being the length and the internal diameter of Tube # i respectively.

The measured split ratio, z_{meas} , was obtained from the measured flow-rate, F_2 , in Tube #2 ($z_{meas} = F_2/F_1$). In the absence of flow splitting (Setting #A), the split ratio was 1. The difference between calculated and measured values in Table 3 may be imputed to internal diameter irregularity.

The peak variance due to flow splitting (σ_{split}^2) was assessed by subtracting the sum of the peak variance measured with Detector #1 (σ_{D1}^2) and that due to Tube #1 ($\sigma_{tubing\ 1}^2$) from the peak variance measured with Detector #2 (σ_{D2}^2), Eq. (14).

$$\sigma_{split}^2 = \sigma_{D2}^2 - \sigma_{D1}^2 - \sigma_{Tub\ #1}^2 \quad (14)$$

403 $\sigma^2_{\text{tubing } 1}$ was measured in the absence of flow splitting with Detector #1 and 2 in series
404 ($\sigma^2_{\text{Tube \#1}} = \sigma^2_{D2} - \sigma^2_{D1}$). It should be noted that σ^2_{D2} corresponds to the total extra-column
405 variance (σ^2_{ext} in Eq. (2)).

406 4.4. Results and discussion

407 Throughout this study, five different settings were studied (Table 3), which differentiated
408 themselves from their measured split ratio and/or their tubing geometry. For the five
409 settings, the dimensions of Tube #1 were the same and hence the variation of $\sigma^2_{\text{Tube \#1}}$ with
410 the total flow-rate (F_1). F_1 was varied in the range 50-700 $\mu\text{L}/\text{min}$, 20-150 $\mu\text{L}/\text{min}$, 50-
411 100 $\mu\text{L}/\text{min}$ and 100-400 $\mu\text{L}/\text{min}$ with settings B, C, D and E respectively. The experimental
412 variation of $\sigma^2_{\text{Tube \#1}}$ with F_1 is given in Fig.4. It could be fitted with an exponential function. As
413 highlighted in Fig.4, the variance increases linearly with the flow-rate up to a value of nearly
414 200 $\mu\text{L}/\text{min}$ and is almost unchanged beyond (i.e. around 8 μL^2). This is in good agreement
415 with reported studies, showing that peak variance values deviate from linearity at high flow-
416 rates with non ideal tubing (i.e. short, coiled and/or rugged tubing) [73].

417 Similarly, the variation of σ^2_{split} with F_1 and hence with F_2 was studied in the presence of the
418 T-union (Fig.5). Two different situations were considered to assess to what extent the peak
419 variance may vary: a decrease in the internal diameter of Tube #2 while keeping the split
420 ratio nearly constant (Setting B and C (Fig.5a)) and a strong decrease in the split ratio while
421 keeping the internal diameter of Tube #2 constant (Setting C, D and E (Fig.5b)).

422 The impact of the internal diameter of Tube #2 was investigated with two different internal
423 diameters, 65 μm (setting B) and 25 μm (setting C). As shown in Fig.5a, the curves,
424 representing the variation of the peak variance with F_2 , are very similar in both cases with
425 only 2 μL^2 difference between both curves, indicating that the internal diameter of Tube #2
426 has little or no impact on the dispersion generated by the split. On the other hand, the
427 impact of the split ratio was found to be very significant as highlighted in Fig.5b. Considering
428 the same resulting F_2 value (e.g. 8 $\mu\text{L}/\text{min}$), the measured peak variance increased from 6 to
429 34 μL^2 with a split ratio decreasing by a factor of about 2 (1:4.8 to 1:9.4), and up to 200 μL^2
430 with a split ratio decreasing by a factor of 10. The impact of the flow splitter on the
431 dispersion can also be visually assessed by comparing the peaks obtained with the different
432 settings. The x-axis is expressed in volume units to have a better comparison of peak

broadening. For a given mobile phase flow-rate of 0.1 mL/min, Fig. 6a shows the degradation of the peak shape when the split ratio decreased from 1:1 (no flow splitting) to 1:50 (only 2 μ L/min sent to UV-Detector 2). In this latter case, the variance due to the split reached 77 μ L². When considering a low F_2 value of 10 μ L/min (Fig.6b), the degradation is even more important with 208 μ L² obtained when the split ratio is decreased down to 1:50. In addition to peak broadening, Fig. 6 clearly points out the problem of sensitivity that can be encountered if the interface and in particular the split device are not carefully chosen.

According to Fig.1a, a total extra-column variance of only 100 μ L² is sufficient to lose more than 70 % of the column plates in UHPLC conditions. Consequently, these results clearly underline the importance of finding the best trade-off between the split ratio which must be high enough, the flow entering ICP-MS which must be low enough and the column flow-rate which must be adapted to UHPLC conditions (typically in the range 400 to 1000 μ L/min). In any case, the additional extra-column dispersion brought by flow splitting should be carefully evaluated. Micro-concentric nebulizers can be considered as a good option for the coupling of UHPLC with ICP-MS since they avoid the need for low split ratios. Such nebulizers usually work with flow-rates of around hundreds μ L/min as those required with narrow bore columns. On the other hand, the use of a flow splitter in association with a total consumption spray chamber operated at around 10 μ L/min, seems inappropriate for UHPLC purposes.

5. Conclusion and future trends

Within the last number of decades, analytical techniques have evolved from the simple determination of the total amount of a metal element to the determination of its chemical species. In speciation studies, ICP-MS is now routinely coupled with liquid chromatography (LC-ICP-MS).

Most applications are performed in an aqueous solvent and are therefore much easier to implement than those needing the introduction of a large amount of organic solvent into the plasma source. Unfortunately, very few analysts pay enough attention to the chromatographic aspects when coupling LC and ICP-MS. During method development, special attention should be focused on extra-column dispersion to minimize it, avoiding excessive dead volume. In view of this article, it clearly appears that efforts need to be made

in this specific area before analyzing complex matrix. Indeed, the benefit of method development performed to enhance the analytical performance (resolution and sensitivity) could be lost if solute dispersion in the interface, located between separation and detection devices, is not minimized. A special attention must be paid to a better description of Sample Introduction System since it seems to be the critical part of the coupling and its characteristics are not always specified by the authors. For speciation analysis, it is highly recommended to thoroughly characterize the interface by evaluating extra column dispersion induced by SIS and ensuring that less than 20 % plates are loss. This will be extensively discussed in Part II.

Flow splitting can be used with total consumption spray chambers to reduce the flow-rate prior to the sample introduction system but it must be sized properly. As shown in this study, a low split ratio can significantly increase solute dispersion and hence solute dilution. Micro-concentric nebulizers are expected to be more appropriate with possible flow-rates of around hundreds $\mu\text{L}/\text{min}$, thus without the need of flow splitting. Such nebulizers will be compared in the second part of this study which will be devoted to the comparison of commercially available Sample Introduction Systems in term of extra-column dispersion.

A last interesting point, in our view, is the impact of extra-column peak broadening on the whole analytical performance when using more complex hyphenated techniques such as 2D-LC-ICP-MS. In the future, if online comprehensive two-dimensional separations are more extensively developed, a particular attention should be paid to the set-up so that every additional dead volume could be assessed in terms of additional dispersion.

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Figure captions

Figure 1: Percentage of remaining plates (β^2) as a function of extra-column variance for different column geometries providing (a) 10000 and (b) 30000 plates. Calculations from Eqs. (6) and (7) with $k_e=3$; $h=3$ and $\epsilon_t=0.7$.

Figure 2: Relative distribution of nebulizers and spray chambers depending on the column inner diameter. Based on 55 reported studies dealing with LC-ICP-MS for organic matrices (from 1995 to 2017)

Figure 3: Schematic representation of the instrument set-up for peak variance measurement. F_1 is the total flow-rate delivered by the pump, F_2 is the flow-rate towards UV Detector 2, and F_3 is the flow-rate sent to the waste. Tubing geometry is given in Table 3.

Figure 4: Variation of the variance due to the tube 1 ($\sigma_{\text{Tube 1}}^2$) as a function of F_1 . See Table 3 for tubing geometry and Fig. 3 for instrument set-up. Solute: methylparaben (75 ppm in 50/50 Water/MeOH v/v). 1 μL injected. No column. Mobile phase: 50/50 water/MeOH v/v. 254 nm.

Figure 5: Variation of the variance due to the split (σ_{split}^2) with F_2 . (a) effect of the tube i.d.

777 (25 μm and 65 μm) considering the same split ratio (1:4.8). (b) effect of the split ratio (1:4.8,
778 1:9.4 and 1:50. Same other conditions as in Fig.4.

779

780 Figure 6 : Overlay of the peaks obtained in Detector 2 with settings A, B, C, D and E. (a) Same
781 F_1 (100 $\mu\text{L}/\text{min}$) resulting in different F_2 (100, 20.8, 20.8, 10.6, 2 $\mu\text{L}/\text{min}$ respectively) and (b)
782 different F_1 (10, 50, 50, 100, 400 $\mu\text{L}/\text{min}$ respectively) resulting in same F_2 (10 $\mu\text{L}/\text{min}$).
783 Measured extra-column variance (σ^2_{ext}) is indicated at peak apex. Same other conditions as
784 in Fig.4.

Table 1. Optimum flow-rates (F_{opt}), column plate number (N_{col}), column variance (σ^2_{col}) depending on column internal diameter (d_i) and particle diameter (d_p). Calculations performed with $k_e = 3$; $D_m = 10^{-9}$ m²/s; $L = 150$ mm; $v = 5$; $h = 3$; $\epsilon t = 0.7$, using Eqs. (5) to

		$d_p = 5 \mu\text{m}$		$d_p = 1.7 \mu\text{m}$	
		$N_{col} = 10000$		$N_{col} = 30000$	
	d_i (mm)	F_{opt} ($\mu\text{L}/\text{min}$)	σ^2_{col} (μL^2)	F_{opt} ($\mu\text{L}/\text{min}$)	σ^2_{col} (μL^2)
Conventional	4.6	698	4867	2052	1655
Narrow bore	2.1	145	211	428	72
Micro bore	1	33	11	97	4
Capillary	0.3	3	0.088	9	0.03
Nano bore	0.075	0.2	0.00034	0.5	0.00012

(12).

Table 2. Reported LC-ICP-MS studies on organic matrices. Evaluation of the percentage of remaining plates (β^2). Calculations with a retention factor of 3, from Eqs. (1) to (12), with S = 4 (small molecules) and S = 50 (large molecules).

[Ref] (Figure)	Sample	LC conditions (Elution mode, column dimension, gradient)	Nebulizer		Spray chamber			β^2 (%)
			Type	Brand	Type	Brand	Cooling	
Conventional column								
[17] (Fig. 3)	Standards, urine, faeces from dog and rat, ^{14}C	RPLC, C18, (250 x 4.6 mm, 5 μm), 1000 $\mu\text{L}/\text{min}$ Gradient (Phase A: 0.1 M ammonium acetate pH 7.5, Phase B: 10 % 1 M ammonium acetate pH 7.5–45 % MeOH–45 % ACN)	Micro-concentric	PFA-LC	Cyclonic	PC ³	2°C	24
([24], Fig 2)	Antituberculosis drug, ^{81}Br	RPLC, C18 (250 x 4.6 mm, 5 μm), 1000 $\mu\text{L}/\text{min}$ Gradient (Phase A: 0.1 mol/L ammonium acetate buffer, Phase B: 10 % 1 mol/L ammonium acetate, 45 % MeOH, 45 % ACN)	Micro-concentric	ES-2050 microflow PFA-LC	Cyclonic	PC ³	2°C	16
[20], Fig 1	Standards Fish, Hg	RPLC, C18 (100 x 4.6 mm, 3.5 μm), 450 $\mu\text{L}/\text{min}$ Isocratic (55 % MeOH + 0.1 % mercaptoethanol + 45 % 60 mM ammonium acetate, pH 4.0)	Micro-concentric	MicroMist	nd	nd	nd	31
([25], Fig 1)*	Urine and bile in rat, ^{127}I	RPLC, C18 (150 x 4.6 mm, 3 μm), 1000 $\mu\text{L}/\text{min}$ Isocratic (30 % ACN + 0.1 % formic acid for analysis of 3- and 4-iodobenzoic acid metabolites or 20 % ACN + 0.1 % formic acid for analysis of 2-iodobenzoic acid metabolites)	Micro-concentric	PFA concentric	Double pass	nd	-7°C	14
([58], Fig 2)	Human hair, fish sample, Hg	RPLC, C18 (150 x 4.6 mm, 5 μm), 800 $\mu\text{L}/\text{min}$ Isocratic (35 % MeOH + 40 % ACN + 25 % water containing 1.0×10^{-4} mol/L)	nd	nd	nd	nd	nd	34
([26], Fig 1)	Standards, Hg	RPLC, C18 (150 x 4.6 mm, 5 μm), 700 $\mu\text{L}/\text{min}$ Isocratic (90 % (v/v) MeOH–10 % (v/v) water containing DDTC 1.0×10^{-4} mol/L)	nd	nd	nd	nd	-5°C	86
Narrow bore column								
([59], Fig 1)	Standard Fish tissue and hair, Hg,	RPLC, C18 (150 x 3.2 mm, 3 μm), 400 $\mu\text{L}/\text{min}$ Isocratic (50 % MeOH + 50 % water (v/v) with 0.01 % 2-mercaptoethanol)	Micro-concentric	Microflow PFA	nd	nd	-5°C	59
([60], Fig 3)	Standards from pharmaceutical companies, Gd,	HILIC, (150 x 2.1 mm, 3 μm), 250 $\mu\text{L}/\text{min}$ Isocratic (Phase A: 50 mmol/L aqueous ammonium formate, Phase B: ACN, 30 % A and 70 % B)	Micro-concentric	PFA microflow ST	Cyclonic	nd	-7°C	<5
([60], Fig 4)					Desolvation unit	APEX Q	-5°C	<5
(66), Fig 2)	Blood plasma, Fe,	HILIC, (150 x 2.1 mm, 3 μm), 250 $\mu\text{L}/\text{min}$ Isocratic (Phase A: 25 mmol/L aqueous ammonium acetate, Phase B: ACN, 30 % A and 70 % B)	Micro-concentric	PFA microflow ST	Cyclonic	nd	-5°C	18
([33], Fig 1)	Standards Xylem sample, ^{54}Fe	HILIC, (150 x 2.1 mm, 5 μm), 100 $\mu\text{L}/\text{min}$ Gradient (Phase A: 10 mM ammonium acetate in water Phase B: 10 mM ammonium acetate in MeOH)	nd	nd	nd	nd	nd	14
([63], Fig 2)	Human serum, ^{202}Hg	RPLC, C5 (150 x 2.1 mm, 5 μm), 300 $\mu\text{L}/\text{min}$ Gradient (Phase A: 0.1 % formic acid in water, Phase B: ACN)	Concentric	Meinhard nebulizer TR-30-A3	nd	PC3	nd	8
([64], Fig 4)	Carbonic anhydrase, thimerosal, ^{202}Hg	RPLC, C18 (150 x 2.1 mm, 5 μm), 300 $\mu\text{L}/\text{min}$ Gradient (Phase A: 0.1 % acid formic in water, Phase B: ACN)	Micro-concentric	PFA MicroFlow	Cyclonic	nd	-5°C	8
([9], Fig 1)	Standards Human cell, Pt	HILIC, (150 x 2.1 mm, 5 μm), 100 $\mu\text{L}/\text{min}$ Isocratic (70 % DMF + 30 % 20 mM ammonium acetate or 70 % ACN + 30 % 20 mM ammonium acetate)	Parallel path	MiraMist	Cyclonic	nd	5°C	11

[Ref] (Figure)	Sample	LC conditions (Elution mode, column dimension, gradient)	Nebulizer		Spray chamber			β ² (%)
			Type	Brand	Type	Brand	Cooling	
([52], Fig 2)	Standards Arsenosugars in algal extract, As	Anion Exchange, (100 x 2.1 mm, 5 μm), 100 μL/min Isocratic (60 mM aqueous ammonium dihydrogen phosphate of pH 5.9)	Micro- concentric	PFA-ST (Dead volume 13 μL)	Cyclonic	Cinnabar (20 mL)	nd	70
([29], Fig 1)	Standards, ⁷⁵ As	HILIC, (50 x 2.1 mm, 3.5 μm), 100 μL/min Isocratic (70 % ACN + 20 % 125 mM ammonium acetate pH 8.3)	Micro- concentric	Micro- concentric	Scott double pass	nd	nd	<5
([19], Fig 4)	Standards, Sn	RPLC, C18 (150 x 2 mm, 5 μm), 200 μL/min Isocratic (70 % (v/v) MeOH + 29 % (v/v) water + 1 % (v/v) glacial acetic acid and 4 mM ion pair reagent (1- pentansulfonic acid sodium salt 1-hydrate))	Nd	nd	nd	nd	nd	17
([28], Fig 1)	Rat hepatocyte , ⁸² Se	RPLC, C18 (250 x 2 mm, 5 μm), 200 μL/min Gradient (Phase A: 0.1 % formic acid 2% MeOH, Phase B: 0.1 % formic acid in 50 % MeOH)	Micro- concentric	MicroMist	Cyclonic	PC3	4°C	50
([47], Fig 5)*	Standards, ¹²⁷ I	RPLC, (250 x 2 mm, 5 μm), 300 μL/min Isocratic (22 % (v/v) ACN ; 0.08 % (v/v) trifluoroacetic acid), Before going to the ICP-MS using make-up solution 2 % (v/v) HNO ₃ ; (0.7 ml/min)	Cross-flow	Gem Tip (Perkin Elmer)	Double pass	Ryto	nd	6
Microbore column								
([31], Fig 3)	Plasma Sample Cisplatin, ¹⁹⁵ Pt	HILIC, (150 x 1 mm, 3 μm), 90 μL/min Gradient (Phase A: 95 % ACN, 5 % water, 0.05 % formic acid, Phase B: 5 % ACN, 95 % water, 0.05 % formic acid	Micro- concentric	PFA-ST nebulizer	Cyclonic	PC3	-5°C	<5
([70], Fig 5)	Fish liver and muscle, Hg, Pb	RPLC, C18 (150 x 1 mm, 5 μm), 120 μL/min Isocratic (0.2 % (v/v) 2-mercaptoethanol, 174.2 mg/L SPS, 12 % (v/v) MeOH and 1 mg/L EDTA, pH 2.8)	Total consumption nebulizer DIHEN-170-AA					<5
([71], Fig 1)	Arsenic species Urine, As	Anion Exchange, (150 x 1 mm, 5 μm), 100 μL/min Isocratic (20 mM mono-ammonium dihydrogen phosphate acid and diammonium hydrogen phosphate buffer)	Total consumption nebulizer DIN (Microneb 2000)					<5
([71], Fig 1)	Arsenic species Urine, As	Anion exchange, (150 x 1 mm, 5 μm), 100 μL/min Isocratic (20 mM mono-ammonium dihydrogen phosphate acid and diammonium hydrogen phosphate buffer)	Micro- concentric	Micro- concentric nebulizer (MCN-100)	Double pass	Scott Ryton	nd	<5
([71], Fig 1)	Arsenic species Urine, As	Anion exchange, 5 (150 x 1 mm, 5 μm), 100 μL/min Isocratic (20 mM mono-ammonium dihydrogen phosphate acid and diammonium hydrogen phosphate buffer)	Cross flow	nd	Double pass	Scott Ryton	nd	<5
([55], Fig 3)	Standards, Hg	RPLC, C18 (50 x 1 mm, 3.5 μm), 100 μL/min Isocratic (Cobalamin: 25 mM ammonium acetate, 10 % ACN, Organomercury and lead: 7.5 mM PIC-B5, 20 % ACN)	Total consumption nebulizer DIHEN (reduce by inserting a 0.008 in id tubing into the nebulizer					<5
	Standards, Pb							
	Standards, Co							
Capillary column								
([67], Fig 5)*	Standards Synthetic phospho- peptide, ²³⁸ U, ¹¹⁵ In, ¹²⁷ I, ³¹ P	RPLC, C18 (250 x 0.3 mm, 5 μm), 116 μL/min Gradient (Phase A: 100/0.065 water/TFA, Phase B: 80/20/0.05 ACN/water/TFA)	Total consumption nebulizer Modified DIHEN					<1
([67], Fig 5)*		RPLC, C18 (250 x 0.3 mm, 5 μm), 116 μL/min Gradient (Phase A: 100/0.065 water/TFA, Phase B: 80/20/0.05 ACN/water/TFA)	Micro- concentric	PFA-LC	Cyclonic	PFA	Nd	<1

nd: not determined or not found in the literature

* : use of flow splitting

Table 3. Tubing geometry and split ratio (calculated and measured) for Setting A, B, C, D and E (see Fig. 3 for instrument set-up). Split ratio were calculated from Eq. (13).

	Setting A	Setting B	Setting C	Setting D	Setting E
Tube 1	60 cm x 100 μ m				
Tube 3	-	62 cm x 100 μ m	44.5 cm x 65 μ m	24 cm x 100 μ m	132 cm x 127 μ m
Tube 2	-	29 cm x 65 μ m	6 cm x 25 μ m	56 cm x 65 μ m	9 cm x 25 μ m
Calculated split ratio	1:1	1:3.6	1:7.4	1:14	1:45
Measured split ratio	1:1	1:4.8	1:4.8	1:9.4	1:50

Figure 1

Column	Nebulizer	Spray Chamber
Conventional (10 studies)	<p>Concentric 10%</p> <p>Not specified 30%</p> <p>Microcentric 50%</p> <p>Hydraulic High Pressure Nebulizer 10%</p>	<p>Double pass 20%</p> <p>Desolvation unit 10%</p> <p>Cyclonic 30%</p> <p>Not specified 40%</p>
Narrow bore (24 studies)	<p>Microcentric 67%</p> <p>Not specified 13%</p> <p>Concentric 4%</p> <p>Cross-flow 4%</p> <p>Parallel path 12%</p>	<p>Double pass 25%</p> <p>Desolvation unit 8%</p> <p>Cyclonic 54%</p> <p>Not specified 13%</p>
Microbore bore (17 studies)	<p>Microcentric 47%</p> <p>Total consumption 35%</p> <p>Not specified 12%</p> <p>Cross-flow 6%</p>	<p>Double pass 18%</p> <p>Not specified 9%</p> <p>Cyclonic 64%</p> <p>Desolvation unit 9%</p>
Capillary (11 studies)	<p>Microcentric 18%</p> <p>Total consumption 18%</p> <p>Not specified 64%</p>	<p>Not specified 56%</p> <p>Cyclonic 44%</p>

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6