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Supercritical Fluid Chromatography – Mass Spectrometry: Recent Evolution And Current Trends

Gioacchino Luca Losacco, Jean-Luc Veuthey, Davy Guillarme

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SUPERCRITICAL FLUID **CHROMATOGRAPHY** MASS 1 SPECTROMETRY: RECENT EVOLUTION AND CURRENT TRENDS 2 3 AUTHORS: Gioacchino Luca Losacco¹, Jean-Luc Veuthey¹, Davy Guillarme^{1*} 4 5 (1) School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CMU-6 7 Rue Michel Servet 1, 1211 Geneva 4, Switzerland 8 *corresponding author 9 **CORRESPONDENCE:** 10 School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CMU -11 Rue Michel-Servet 1, 1211 Geneva 4, Switzerland 12 Phone: +41 22 379 34 63 13 E-mail: Davy.guillarme@unige.ch 14 15 **KEYWORDS**: 16 Supercritical fluid chromatography; interfaces; matrix effects; sensitivity; doping agents; 17 metabolomics 18 19

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23 HIGHLIGHTS:

- A detailed summary on SFC-MS interfaces is given, with emphasis on current issues
 and potential solutions.
- Differences between LC-MS and SFC-MS in terms of matrix effects generated are
 highlighted.

Sensitivity under SFC-MS has been demonstrated to be comparable to what it can be reached in LC-MS conditions.

- Applications for SFC-MS are shifting towards the analysis of compounds with
 increasing polarity and analytes available in complex matrices.

ABSTRACT

Supercritical fluid chromatography (SFC) has recently experienced renovated impulse from research groups. Its hyphenation to mass spectrometers (MS) proved to be of significant importance in catalysing interest from researchers. In contrast to liquid chromatography (LC), the coupling of SFC-MS requires the use of an interface in order to deal efficiently with the decompression of supercritical CO₂ and possible precipitation issues of samples while entering the ionization chamber. The most common SFC-MS interfaces employ an additional sheath pump that reduces sample precipitation. However, there are still issues in dealing with the CO₂ decompression phenomenon, with different solutions being given. Matrix effects (MEs) under SFC-MS have proved to be quite different from those generally observed in LC-MS, with ion suppression being the main form of ME. Nonetheless, SFC-MS is capable of reaching comparable sensitivity values to LC-MS, and in some cases performing even better. Several applications have been recently developed for SFC-MS, spacing from the analysis of plant extracts, biological matrices for anti-doping and forensic purposes, as well as highly polar compounds such as carbohydrates and endogenous metabolites.

70 **1. Introduction**

The use of mass spectrometers (MS) as a detector hyphenated to chromatographic separation has known an incredible growth, in the recent years, thanks to its high versatility, sensitivity, and range of possible applications [1, 2]. Considering the diversity of MS analyzers present on the market (i.e. single and triple quadrupole, time of flight, ion trap, Orbitrap and hybrid instruments), it is possible to perform qualitative and quantitative analysis at very high sensitivity [3-5], as well as to generate elevated MS resolution between compounds having very similar mass-to-charge values [6, 7].

One of the most successful marriages between chromatography and mass spectrometry is 78 now represented by the hyphenation of liquid chromatography (LC) with MS [8-10]. This 79 coupling became possible thanks to the development of atmospheric pressure ionization 80 (API) sources such as electrospray ionization (ESI) [11, 12] and atmospheric pressure 81 chemical ionization (APCI) [13]. Besides LC, other separation techniques have also been 82 successfully hyphenated to MS, including gas chromatography (GC) [14] capillary 83 electrophoresis (CE) [15], and supercritical fluid chromatography (SFC) [16, 17]. SFC was 84 initially developed during the 1960s and regained the attention of several research groups 85 starting from the 1980s [18], but the interest remained limited to chiral separation [19] and 86 preparative chromatography [20], due to a lack of robustness and sensitivity of the 87 instrumentation [21]. Since 2012, a new generation of SFC instruments was introduced on 88 the market. These new systems possess various desirable features, such as i) reduced 89 system volume and a relatively high upper-pressure limit, compatible with columns packed 90 with sub-2 µm particles, ii) improved robustness and iii) easy MS hyphenation [22]. This 91 92 allows SFC to transition into ultra-high performance supercritical fluid chromatography 93 (UHPSFC) [18], in a similar way to what has been witnessed with LC since 2004, with a consequent increase in terms of interest and publications being made. A quick analysis 94 made on the main research platforms currently available shows a gradual but constant 95 increase in articles which have keywords such as "supercritical fluid chromatography" or 96

"SFC", in the period from 2012 (343 publications) till 2018 (634 publications). This 97 chromatographic technique has the peculiarity of employing a supercritical (or often 98 99 subcritical) mobile phase, thanks to the use of carbon dioxide in its supercritical state as major constituent [18, 23]. In modern UHPSFC, carbon dioxide is always mixed with an 100 organic modifier, usually methanol, which ensures the complete elution of compounds from 101 low to high polarity [23]. Some salts (i.e., ammonium formate, ammonium hydroxide...), as 102 103 well as acids (i.e. formic acid, trifluoroacetic acid...) or bases (i.e. ammonium hydroxide, 104 diethyl- and triethylamine...), and a small amount of water could also be added to the mobile phase to improve method repeatability and peak shapes of ionizable substances. Finally, the 105 use of a supercritical mobile phase presents several advantages in chromatography, 106 including a minor environmental impact compared to organic solvents such as n-hexane or n-107 108 heptane, low viscosity, high diffusion coefficients, and high density, thus enabling SFC to combine the advantages of LC and GC [18, 23]. 109

The aim of this review was to describe the latest developments related to the hyphenation of 110 UHPSFC and MS, highlighting some advantages that this technique can offer in contrast with 111 the current state-of-the-art techniques. First, a detailed description of the UHPSFC-MS 112 interfaces available on the market will be provided, including some potential issues related to 113 the use of a supercritical fluid. Secondly, the influence of the make-up solvent nature and the 114 evaluation of matrix effects will be assessed. Then, a comparison of achievable sensitivity in 115 116 UHPSFC-MS and UHPLC-MS will be performed. Finally, an overview of some relevant applications that have been developed in the last few years will also be given. 117

118 2. UHPSFC-MS interfaces

The hyphenation of UHPSFC with MS is not as straightforward as with LC/UHPLC instruments. Indeed, supercritical fluids possess much higher compressibility than liquids, that needs to be controlled, particularly when the fluid is not anymore under the backpressure control [24]. Indeed, when the pressure is released, analytes can precipitate before entering the MS instrument. Besides the regulation of backpressure, the interface

should also help to improve the ionization yield in ESI, particularly when the mobile phase is composed of a high proportion of CO₂. Lastly, the chromatographic integrity (retention, selectivity, and efficiency) should also be maintained when MS detection is used. For all these reasons, the providers of SFC instruments have developed several interface schemes over the years (Fig. 1), able to solve these different issues [24, 25].

The most common interface available on the market is known as the "pre-BPR splitter with 129 sheath pump", commercialized by Waters and Agilent [25] (see Fig. 1D). This interface 130 131 consists of two zero-dead-volume (ZDV) T-unions linked in series, allowing the addition of a make-up solvent from a sheath pump (first ZDV T-union) and the use of an active-132 backpressure regulator (ABPR) (second ZDV T-union), to direct only a limited part of the 133 flow-rate into the MS ionization source, while the remaining part goes to the waste. This 134 interface offers the obvious advantage of reducing the possible precipitation of samples in 135 the mobile phase. In addition, thanks to the flexible BPR regulation and the presence of the 136 sheath pump, it also allows sending a highly suitable mobile phase flow rate and composition 137 to the ESI sources, thus producing an excellent sensitivity [25]. 138

The second available interface, among the most popular ones, is called "BPR and sheath 139 pump with no splitter", commercialized by Shimadzu and Agilent (see Fig. 1E). In this 140 configuration, there is only one ZDV T-union, used to deliver the make-up solvent. This 141 interface, which does not possess flow-splitting, is well suited for APCI-MS, which is a mass 142 143 flow dependent device, since it delivers the entire sample to the MS [24]. Moreover, the last tubing entering into the ionization source passes through the BPR, which is heated at a 144 relatively high temperature (around 50°C), to limit decompression cooling phenomenon and 145 solute precipitation. Until now, this interface has been rarely employed for real applications, 146 and therefore its advantages and drawbacks are still not well identified [25]. 147

A remark has to be done on the Agilent SFC-MS interface, since it is the only one that allows the user to choose between the "*pre-BPR splitter with sheath pump*" and the "*BPR and sheath pump with no splitter*" configurations.

Besides these two interfaces, there are also a few other solutions that have been described for hyphenating UHPSFC and MS, but they present some major issues, making them inferior to the ones previously described. More details on the different interfaces currently available can be found in a recently published review from our group [25].

When hyphenating UHPSFC and MS, several important issues need to be considered [24-155 26]. In the two previously described interfaces, the BPR module is located before the MS. 156 Therefore, there is no control over the mobile phase state entering the ionization chamber. In 157 this part of the setup, the CO₂ is not under the influence of the BPR and should decompress 158 endothermically, which leads up to different problems [24]. First, the decompression, 159 followed by a drop in the temperature at the connector level, increases the risk of analyte 160 precipitation [24, 25]. Moreover, the addition of the make-up solvent, necessary to replace 161 decompressed CO₂, might be insufficient to ensure the solubility of the samples, leading to 162 possible precipitation issues [27]. Another issue related to the uncontrolled CO₂ 163 decompression is the possible peak broadening that has been previously reported [24]. This 164 phenomenon could be attributed to different factors: the temperature drop is certainly one of 165 166 them, since it increases solvent viscosity and thus reduces analytes diffusion coefficient [24]. In addition, considering that there is no pressure and temperature control in the tubing 167 located after the BPR, phase separation is most likely to occur between the liquid organic 168 modifier and gaseous CO₂ [24, 25, 27]. As described elsewhere [24], to better understand 169 170 the influence of the phenomena described above, it is advised to follow the vapor-liquid equilibrium (VLE) curves for CO_2 +methanol mixtures. Different situations can be foreseen: 171 172 the flow patterns can greatly change, with the formation of CO₂ bubbles of different diameters based on the volume ratios between the gas and the liquid. This, consequently, affects the 173 linear velocity of the flow entering the MS. Linear velocity is also influenced by the change in 174 surface tension, viscosity and other parameters which are not behaving as expected [24]. 175 The phase separation can be another potential problem that should not be underestimated, 176

especially since it might lead to more severe issues such as band broadening or even loss ofthe chromatographic separation.

179 3. Different solutions have been found to tackle these drawbacks observed in the SFC-MS setup. For the precipitation issue, the addition of a sheath pump, which 180 continuously delivers a make-up solvent (i.e., methanol, methanol + buffer, methanol + 181 small amount of water), was found to be a good solution [27]. Indeed, the delivery of a 182 methanol-rich solvent strongly limits the precipitation of polar compounds, without 183 sacrificing too much SFC and MS performance. The addition of a make-up solvent, 184 however, may lead to the insurgence of another potential problem: a dilution factor can 185 appear which could negatively affect MS sensitivity, especially on concentration-186 dependent ionization sources such as ESI [25, 27-29]. Contrary to what could be 187 expected, the dilution factor remains always reasonable, whatever the mobile phase 188 and make-up conditions, thanks to the use of the active BPR [27, 30]. Regarding the 189 management of CO₂ decompression, a solution is to modify the interface [6, 31]. One 190 key parameter is obviously the temperature that needs to be controlled, to avoid phase 191 separation. As described in more details elsewhere [24], heating is not always the best 192 choice. The use of combined isenthalpic and isopycnic plots, for mixtures of 193 CO₂/methanol with fixed compositions, clearly highlight that cooling, instead of heating, 194 should be preferred [24]. Indeed, the analysis of these plots definitely indicates that, by 195 lowering the temperature, it is possible to avoid the area in which phase separation 196 occurs for a greater range of pressure values [24]. The temperature reduction, 197 198 therefore, translates into a wider range of the CO_2 decompression. Density, also, does not change, which therefore translates in much fewer precipitation issues of several 199 200 compounds, which were soluble with a high-density mobile phase. The other parameter is the interface geometry; indeed, changing the geometry of the capillaries 201 used in the interface (i.e., length, inner diameter, etc.) can be an easy solution to 202 maintain a constant mobile phase density. Only two papers, however, [6, 31] describe 203

204 the evaluation of a new capillary restrictor for ESI interfaces, able to reduce the 205 pressure drop in the connector. According to the authors, the new interface design has 206 brought a more stable ESI spray, positively affecting the peak shapes and 207 repeatability, thus allowing a better quantification of the compounds that have been 208 tested [6, 31]. Matrix effects in SFC-MS vs. LC-MS

209 UHPLC coupled to ESI-MS and tandem ESI-MS/MS instruments, is one of the most 210 successful analytical techniques for the analysis of endogenous and exogenous compounds in complex matrices, such as urine, plasma or plants extract [32-34]. However, when 211 analyzing biological matrices, it is important to consider the possible enhancement or 212 suppression of analytes signals in the ionization stage by compounds that are present in the 213 matrix, and co-elute with the investigated compounds [35, 36]. This effect, better known as 214 215 the matrix effect (ME), negatively affects the quantification of substances present in such matrices. Indeed, a signal suppression or enhancement of targeted substances has an 216 obvious impact on LODs/LOQs and may increase variability on peak areas. Therefore, 217 validation of the analytical method can become challenging. Since the retention mechanism 218 in SFC on polar stationary phases (mostly polar interactions) is orthogonal to LC (mainly 219 hydrophobic interactions), coelution of investigated compounds and substances contained 220 within the matrix may be very different. Therefore, UHPSFC-ESI-MS(/MS) can be considered 221 as a useful strategy to minimize or at least modify the impact of ME, in comparison with 222 UHPLC-ESI-MS(/MS) [37]. 223

In the last 3-4 years, there has been an increasing number of studies dealing with the application of UHPSFC-MS for the analysis of biological matrices [38-42]. Urine has been by far the most widely used matrix, due to its relative easiness of collection and sample treatment. In the case of urine, ME is mainly due to the presence of polar compounds such as urea, creatinine, glucuronic acid, uric acid, etc., as well as salts. Svan *et al.* [36] have recently made a systematic comparison of ME between RPLC-MS and SFC-MS, using 11 representative drugs in urine samples. In their study, ME was evaluated using the post-

column infusion matrix profiles approach. To explain the differences observed in terms of 231 ME, the authors first described the modification in separation profiles of matrix components 232 233 between the two chromatographic techniques. Indeed, compounds generating ME in urine, which are highly polar, are eluted quite early in RPLC conditions, while they are strongly 234 retained on SFC conditions and lately eluted thanks to the increasing concentration of the 235 polar organic modifier in the mobile phase [36]. The differences, however, are not limited 236 237 only to the separation profiles. In fact, under SFC conditions, there is a clear predominance of the ion suppression phenomenon, whose origin was further investigated in a follow-up 238 paper [43]. In RPLC, both types of MEs (ion suppression and enhancement) co-exist, 239 depending on the investigated analyte [36]. A second paper [37] correlated MEs obtained in 240 RPLC and SFC using two different sample preparation methodologies (non-selective and 241 selective), and the Matuszewski's approach was used as the ME evaluation. The conclusions 242 reached by both authors were similar, clearly stating that signal suppression is the major type 243 of ME in SFC for urine [37]. Moreover, SFC has proved to give less ME than RPLC in all 244 245 experiments with urine samples [37]. This statement is further confirmed in other papers, where ME was found to be quite low in SFC-MS conditions [38, 44, 45]. 246

While using plasma, however, the situation seems to be different. Indeed, the ME generated 247 by plasma for around 40 representative drugs in SFC and RPLC [37] gave unexpected 248 results. Higher signal suppression was observed in RPLC vs. SFC with the selective sample 249 preparation methodology (solid phase extraction, SPE). However, the impact of ME was also 250 highly dependent on the selected column chemistry in SFC [37]. In another study, the use of 251 252 protein precipitation (PP) for plasma sample brought results that are similar to urine, with signal suppression being more common in SFC [36]. A third paper dealing with the 253 254 application of SFC for the determination of three major antiepileptic drugs in plasma reports the level of ME around 95-100%, with only one compound subjected to slight signal 255 suppression, stating therefore that SFC does not present issues with ME in plasma [46]. To 256 draw some reliable conditions on ME for plasma samples, there is, however, a need for more 257

experimental results and discussion, due to the limited number of applications reported with human plasma under SFC conditions. In addition, it is also important to keep in mind that ME may be highly dependent on the geometry of the electrospray ionization source.

261

4. Achievable sensitivity in SFC-MS vs. LC-MS

SFC has always been considered as a well-suited technique for MS detectors, thanks to the 262 hybrid nature of the mobile phase, and the use of organic solvents (mostly methanol) with 263 264 higher volatility than water, thus positively influencing the ionization process, especially in 265 ESI mode. The recent introduction of modern and reliable UHPSFC-MS systems allowed to experimentally prove some of the potential benefits of SFC over LC. Indeed, as shown in [28, 266 47-50], excellent values for LODs and LOQs were met, with LOD values often down to below 267 1 ppb [28]. However, SFC-MS does not systematically provide a clear advantage over LC-268 269 MS in terms of sensitivity. Indeed, it was found that, while with the older generation of MS instruments, SFC generally provides a higher sensitivity than LC, with the more recent mass 270 spectrometers, SFC and LC were found to give very close results (Fig. 2) [51]. This 271 observation was explained by the use of improved ionization sources on the more recent MS 272 instruments, making them more able to handle higher proportion of water [51]. As an 273 example, it was found that, out of 43 anabolic agents tested in human urine, LC provided a 274 sensitivity level equal to 0.1 ng/mL for 98% of the analyzed compounds, while in SFC this 275 percentage was reduced to 76% [52]. A similar result was obtained for vitamin D metabolites, 276 277 with worse LLOQs in SFC than LC [53]. The main reason for these negative results is related to the limited injection volume in SFC. Indeed, it is well known that a lower injection volume 278 has to be used in SFC vs. LC, especially when using polar and polar protic solvents such as 279 280 methanol or water as the injection solvents [53-55], which should obviously negatively affect 281 sensitivity. Moreover, different column geometries are generally used in LC and SFC (2.1 mm and 3.0 mm as internal diameters, respectively), which could further increase the dilution 282 factor in SFC and reduce achievable sensitivity [52]. 283

As previously discussed, there is a need to use a make-up solvent to couple SFC with MS. 284 This means that users have the possibility to modify the mobile phase composition before 285 286 entering MS detection, so that the ionization process can be enhanced, especially in ESI 287 mode. Some authors have recently demonstrated how the addition of either small quantities of water or the use of additives/buffers in the make-up pump, increased the MS signals, thus 288 improving sensitivity [28, 48]. Using a wide range of endogenous steroids, the authors 289 290 screened different buffers/additives in the make-up solvent, finding that either pure ammonium fluoride or ammonium fluoride mixed with formic acid in the solvent, can greatly 291 improve ionization efficiency in ESI mode for steroids. In another work, it has been 292 highlighted how the make-up solvent can positively influence the ionization of protease 293 inhibitors in ESI conditions, with a simple tuning of its composition [27]. The authors have 294 concluded that, while in LC, the mobile phase composition is not easily modifiable to 295 enhance MS performance, the necessary addition of the make-up solvent in SFC can 296 297 generate large MS signals increases, also allowing the possibility of considering post-column 298 derivatization to improve further MS detection [27].

299 5. Applications of SFC-MS

As already observed for SFC-UV, there has been a constant and impressive increase in the number of new applications recently developed in SFC-MS.

An important field of application is the analysis of natural products. Indeed, there have 302 already been developments and successful implementations in the past, however, now the 303 constantly growing use of high resolutions MS instruments (HRMS), hyphenated not only to 304 LC but also to SFC, has pushed the latter even further in this area. Besides the analysis of 305 306 lipophilic compounds including lipids in plants [56-59], there is an interesting and growing 307 trend, namely the analysis of compounds with increasing polarity, such as monosaccharides 308 [60], saponins [61] and flavonoids [62]. Other natural compounds are also being analyzed under SFC-MS, such as plant metabolites with interesting potential as drugs (Fig. 3) [63, 64]. 309 310 A specific category, which also attracts attention, is cannabinoids; indeed, the use of this

class of compounds is rapidly increasing, in both medical and forensic applications [65-67]. Today, SFC-MS can be considered as a complementary technique to LC-MS, with an interesting ability in obtaining resolution of positional isomers and diastereomers, with a high degree of orthogonality to LC [68]. Moreover, the methods developed in SFC-MS also fit well with quality control requirements of real-life cannabis samples analysis [49], thanks to an easier sample preparation phase and a robust, fast and generic analytical method [49].

317 A second application area that is being under constant development is the implementation of SFC-MS in the forensic and anti-doping control analysis. Indeed, there has been an 318 important number of papers recently released and focusing on several classes of 319 compounds: amphetamines [45, 69, 70], stimulants and sympathomimetic drugs [51, 71, 72] 320 or anabolic agents and steroids [44, 54, 73, 74] (Fig. 4). Researchers involved in the field of 321 322 anti-doping analysis are now testing new analytical techniques (such as SFC-MS), to find possible advantages to the current state of the art represented by LC-MS. Furthermore, SFC 323 is not only being used as an analytical method but also employed in the sample preparation 324 stage [74], with the aim to replace older methods employed in the sample treatment. 325 Obviously, SFC-MS methods that wish to be employed in anti-doping laboratories also have 326 to be validated. This aspect is being currently investigated by several authors, with a growing 327 number of publications [38, 39, 45, 47, 49, 75] showing that the validation procedure in SFC-328 MS yields similar, if not even better results than LC-MS. Indeed, during different validation 329 processes of SFC-MS methods, it was found that SFC-MS manages to provide better results 330 in terms of identification, reproducibility, precision and accuracy when compared to LC-MS 331 332 [47, 75]. These findings are extremely important in establishing SFC-MS itself as a technique that is compatible with regulated bioanalytical laboratories. 333

Another arising trend in SFC-MS applications is the analysis of hydrophilic and highly hydrophilic compounds under subcritical conditions [40, 76]. SFC has been historically considered as a substitute technique to normal phase LC, and therefore, it has been mostly used for the analysis of compounds with low to medium polarity. However, thanks to the

development of innovative strategies, such as the addition of small amount of water and/or 338 salts in the organic co-solvent, as well as the use of gradient conditions up to 70-100% 339 340 organic modifier, the range of analyzable molecules can be extended to molecules possessing log P values below 0 [76]. Thanks to this new possibility, SFC-MS is now shifting 341 towards the analysis of compounds that classically fall under the domain of HILIC-MS. As 342 example, SFC-MS is now increasingly employed in the field of metabolomics, [76] in 343 particular for the analysis of amino acids [40, 77] and carbohydrates [76, 78]. In addition, due 344 345 to the high versatility of SFC-MS, it can be successfully employed for the simultaneous analysis of both hydrophilic and lipophilic molecules, from carbohydrates to lipids in 346 metabolomics [76] (Fig. 5), from water to fat-soluble vitamins in food [79], and from highly 347 hydrophilic to lipophilic trace organic compounds in environmental samples [80]. As more 348 applications involving the use of SFC-MS with polar and highly polar compounds are arising, 349 it can be stated that SFC-MS has now become a well-suited technique not only for lipophilic 350 compounds, but also for those analytes whose polarity falls between $-2 < \log P > 2$. A recent 351 352 review on the latest applications developed in SFC-MS for natural products, food and environmental analysis as well as bioanalysis and metabolomics is now available [81]. 353

354 In contrast to LC, SFC instruments also offer the possibility to have an online extraction unit linked to the chromatographic system (online SFE-SFC). It is now commercially available 355 and has recently been successfully employed in analytical laboratories in different areas, 356 357 from the metabolic profiling of drugs metabolites in human urine [38] to the determination of carotenoids and apocarotenoids in human blood [82], and the analysis of polycyclic aromatic 358 359 hydrocarbons in soil [83]. In these different studies, the authors highlight the very low sample amounts requirement, possibility to achieve fast analysis and how it has been possible to 360 361 validate those methods [38, 82, 83]. This type of online SFE-SFC instrument, although it still needs to be more deeply characterized, in particular in terms of connections between the 362 extraction, separation, and detection [83], possesses an impressive potential for the analyses 363

364 where sample preparation stages can be time-consuming and do not provide sufficient 365 yields.

366 **6.** Conclusions

The hyphenation of SFC to MS has undoubtedly known an impressive growth in the last five years. The development of several SFC-MS geometry interfaces has enabled to couple both systems, as well as a discrete handling of the supercritical fluid once the mobile phase is not under the influence of the APBR module. There are, however, still aspects that necessitate to be thoroughly covered to understand the influence of the CO₂ decompression and how to better solve issues related to this phenomenon.

373 SFC-MS is increasingly being used to analyze compounds present in biological matrices, 374 from urine to plasma, as well as natural substances available in plant extracts. Matrix effects 375 due by biological samples and their impact on the MS signal and performance have been 376 demonstrated to be quite different from what was observed in LC-MS conditions, offering a 377 good complementarity between those two techniques. Nonetheless, there is still a need to 378 further investigate this aspect, with additional sample preparation approaches and different 379 matrices.

ME also impacted sensitivity in a different way than LC-MS, due to the higher probability of signal suppression, rather than enhancement under SFC-MS conditions. Sensitivity, in general, was found to be in several cases at the same level as in LC-MS, if not even higher. However, there are problems related to the limited injection volume and higher dilution factors that, sometimes, make it difficult for SFC-MS to reach LOQ and LOD values obtained in LC-MS.

Finally, the investigation of the most recent applications clearly shows that SFC-MS is moving towards the analysis of small molecules with increasing polarity. This translates in an increasing overlap with RPLC and HILIC. Indeed, the impressive flexibility of SFC in analyzing compounds within an extremely wide polarity range is probably one of the main

interests behind this technique. Its complementarity to RPLC drives an increasing number of
 research groups, and analytical laboratories are starting to use it, to tackle challenging
 separations achieved under LC-MS conditions.

394 **7. References**

[1] T. Kind, H. Tsugawa, T. Cajka, Y. Ma, Z.J. Lai, S.S. Mehta, G. Wohlgemuth, D.K.
Barupal, M.R. Showalter, M. Arita, O. Fiehn, Identification of small molecules using accurate
mass MS/MS search, Mass Spectrometry Reviews, 37 (2018) 513-532.

A.H. Zhang, H. Sun, X.J. Wang, Mass spectrometry-driven drug discovery for
 development of herbal medicine, Mass Spectrometry Reviews, 37 (2018) 307-320.

[3] N. de Kock, S.R. Acharya, S.J.K.A. Ubhayasekera, J. Bergquist, A Novel Targeted
Analysis of Peripheral Steroids by Ultra-Performance Supercritical Fluid Chromatography
Hyphenated to Tandem Mass Spectrometry, Scientific Reports, 8 (2018) 16993.

403 [4] P. Caron, V. Turcotte, E. Lévesque, C. Guillemette, An LC-MS/MS method for 404 quantification of abiraterone, its active metabolites D(4)-abiraterone (D4A) and 5 α -405 abiraterone, and their inactive glucuronide derivatives, Journal of Chromatography B, 1104 406 (2019) 249-255.

407 [5] J. Roosendaal, K. Wang, H. Rosing, L. Lucas, A. Gebretensae, A. Oganesian, J.H.M. 408 Schellens, J.H. Beijnen, Z. Lin, Development and validation of LC-MS/MS methods for the 409 quantification of the novel anticancer agent guadecitabine and its active metabolite 410 β decitabine in human plasma, whole blood and urine, Journal of Chromatography B, DOI 411 https://doi.org/10.1016/j.jchromb.2019.01.011(2019).

[6] O. Ciclet, D. Barron, S. Bajic, J.-L. Veuthey, D. Guillarme, A. Grand-Guillaume Perrenoud,
Natural compounds analysis using liquid and supercritical fluid chromatography hyphenated
to mass spectrometry: Evaluation of a new design of atmospheric pressure ionization source,
Journal of Chromatography B, 1083 (2018) 1-11.

[7] G. Hermann, M. Schwaiger, P. Volejnik, G. Koellensperger, 13C-labelled yeast as internal
standard for LC–MS/MS and LC high resolution MS based amino acid quantification in
human plasma, J Pharmaceut Biomed, 155 (2018) 329-334.

[8] B. Petrie, M.D. Camacho Muñoz, J. Martín, Stereoselective LC–MS/MS methodologies for
environmental analysis of chiral pesticides, TrAC Trends in Analytical Chemistry, 110 (2019)
249-258.

[9] C. Marchioni, I.D. de Souza, V.R. Acquaro, J.A. de Souza Crippa, V. Tumas, M.E.C.
Queiroz, Recent advances in LC-MS/MS methods to determine endocannabinoids in
biological samples: Application in neurodegenerative diseases, Analytica Chimica Acta, 1044
(2018) 12-28.

[10] W. Lv, X. Shi, S. Wang, G. Xu, Multidimensional liquid chromatography-mass
spectrometry for metabolomic and lipidomic analyses, TrAC Trends in Analytical Chemistry,
DOI https://doi.org/10.1016/j.trac.2018.11.001(2018).

[11] T. Higashi, M. Akaishi, M. Yokota, T. Suzuki, S. Ogawa, Y. Sugiura, T. Nishikawa, K.
Nishimoto, M. Suematsu, A method for determination of aldosterone in adrenal tributary
venous serum by derivatization using Girard P reagent isotopologues followed by LC/ESIMS/MS, Journal of Chromatography B, 1092 (2018) 106-113.

[12] A.-S. Claeson, S. Gouveia-Figueira, H. Stenlund, A.I. Johansson, A standardized
protocol for comparable analysis of GSH/GSSG by UHPLC-ESI-MSMS for human plasma,
Journal of Chromatography B, 1104 (2019) 67-72.

[13] K. Hu, Y. Li, R. Ding, Y. Zhai, L. Chen, W. Qian, J. Yang, A simple, sensitive, and highthroughput LC-APCI-MS/MS method for simultaneous determination of vitamin K1, vitamin
K1 2,3-epoxide in human plasma and its application to a clinical pharmacodynamic study of
warfarin, Journal of Pharmaceutical and Biomedical Analysis, 159 (2018) 82-91.

[14] S. Biswas, R. Mondal, A. Mukherjee, M. Sarkar, R.K. Kole, Simultaneous determination
and risk assessment of fipronil and its metabolites in sugarcane, using GC-ECD and
confirmation by GC-MS/MS, Food Chemistry, 272 (2019) 559-567.

[15] P. Fang, J.-Z. Pan, Q. Fang, A robust and extendable sheath flow interface with minimal
dead volume for coupling CE with ESI-MS, Talanta, 180 (2018) 376-382.

[16] S. Yin, Y. Yang, L. Wu, Y. Li, C. Sun, Recent advances in sample preparation and
analysis methods for vitamin D and its analogues in different matrices, TrAC Trends in
Analytical Chemistry, 110 (2019) 204-220.

[17] X. Zhang, X. Ding, J. Wang, B. Dean, Supercritical fluid chromatography-tandem mass
spectrometry for high throughput bioanalysis of small molecules in drug discovery, J
Pharmaceut Biomed, 164 (2019) 62-69.

- [18] D.P. Poe, Chapter 2 Theory of Supercritical Fluid Chromatography, in: C.F. Poole (Ed.)
 Supercritical Fluid Chromatography, Elsevier2017, pp. 23-55.
- [19] K.L. Williams, L.C. Sander, Enantiomer separations on chiral stationary phases in
 supercritical fluid chromatography, J. Chromatogr. A, 785 (1997) 149-158.
- [20] C. Berger, M. Perrut, Preparative supercritical fluid chromatography, J. Chromatogr. A,
 505 (1990) 37-43.
- [21] T.A. Berger, Chapter 7 Evolution of Instrumentation for Analytical Scale Supercritical
 Fluid Chromatography**This chapter draws upon large sections of TA Berger,
 "Instrumentation for supercritical fluid chromatography," J. Chromatogr. A, 1421 (November
 2015), 171–183. Reproduced with permission from Elsevier. ©Elsevier 2015, in: C.F. Poole
 (Ed.) Supercritical Fluid Chromatography, Elsevier2017, pp. 173-212.
- 462 [22] A. Grand-Guillaume Perrenoud, J.-L. Veuthey, D. Guillarme, The use of columns packed
 463 with sub-2 µm particles in supercritical fluid chromatography, TrAC Trends in Analytical
 464 Chemistry, 63 (2014) 44-54.
- [23] U. Jumhawan, T. Bamba, Chapter 16 Supercritical Fluid Chromatography, in: F. PenaPereira, M. Tobiszewski (Eds.) The Application of Green Solvents in Separation Processes,
 Elsevier2017, pp. 483-516.
- 468 [24] A. Tarafder, Designs and methods for interfacing SFC with MS, Journal of 469 Chromatography B, 1091 (2018) 1-13.
- [25] D. Guillarme, V. Desfontaine, S. Heinisch, J.-L. Veuthey, What are the current solutions
 for interfacing supercritical fluid chromatography and mass spectrometry?, Journal of
 Chromatography B, DOI https://doi.org/10.1016/j.jchromb.2018.03.010.
- 473 [26] V. Desfontaine, J.L. Veuthey, D. Guillarme, Chapter 8 Hyphenated Detectors: Mass
 474 Spectrometry, in: C.F. Poole (Ed.) Supercritical Fluid Chromatography, Elsevier2017, pp.
 475 213-244.
- [27] L. Akbal, G. Hopfgartner, Effects of liquid post-column addition in electrospray ionization
 performance in supercritical fluid chromatography–mass spectrometry, J. Chromatogr. A,
 1517 (2017) 176-184.
 - 19

[28] M.K. Parr, B. Wüst, J. Teubel, J.F. Joseph, Splitless hyphenation of SFC with MS by
APCI, APPI, and ESI exemplified by steroids as model compounds, Journal of
Chromatography B, 1091 (2018) 67-78.

[29] Y. Fujito, Y. Hayakawa, Y. Izumi, T. Bamba, Importance of optimizing chromatographic
conditions and mass spectrometric parameters for supercritical fluid chromatography/mass
spectrometry, J. Chromatogr. A, 1508 (2017) 138-147.

[30] J. Duval, C. Colas, V. Pecher, M. Poujol, J.-F. Tranchant, E. Lesellier, Hyphenation of
ultra high performance supercritical fluid chromatography with atmospheric pressure
chemical ionisation high resolution mass spectrometry: Part 1. Study of the coupling
parameters for the analysis of natural non-polar compounds, J. Chromatogr. A, 1509 (2017)
132-140.

[31] F. Petruzziello, A. Grand-Guillaume Perrenoud, A. Thorimbert, M. Fogwill, S. Rezzi,
Quantitative Profiling of Endogenous Fat-Soluble Vitamins and Carotenoids in Human
Plasma Using an Improved UHPSFC-ESI-MS Interface, Analytical Chemistry, 89 (2017)
7615-7622.

494 [32] J. Aszyk, H. Byliński, J. Namieśnik, A. Kot-Wasik, Main strategies, analytical trends and
495 challenges in LC-MS and ambient mass spectrometry–based metabolomics, TrAC Trends in
496 Analytical Chemistry, 108 (2018) 278-295.

497 [33] V. Avataneo, A. D'Avolio, J. Cusato, M. Cantù, A. De Nicolò, LC-MS application for
498 therapeutic drug monitoring in alternative matrices, J Pharmaceut Biomed, 166 (2019) 40-51.

[34] S. Wang, P. Qi, S. Di, J. Wang, S. Wu, X. Wang, Z. Wang, Q. Wang, X. Wang, C. Zhao,
Q. Li, Significant role of supercritical fluid chromatography - mass spectrometry in improving
the matrix effect and analytical efficiency during multi-pesticides residue analysis of complex
chrysanthemum samples, Analytica Chimica Acta, 1074 (2019) 108-116.

[35] P.J. Rudzki, E. Gniazdowska, K. Buś-Kwaśnik, Quantitative evaluation of the matrix
effect in bioanalytical methods based on LC–MS: A comparison of two approaches, J
Pharmaceut Biomed, 155 (2018) 314-319.

[36] A. Svan, M. Hedeland, T. Arvidsson, C.E. Pettersson, The differences in matrix effect
between supercritical fluid chromatography and reversed phase liquid chromatography
coupled to ESI/MS, Analytica Chimica Acta, 1000 (2018) 163-171.

509 [37] V. Desfontaine, F. Capetti, R. Nicoli, T. Kuuranne, J.-L. Veuthey, D. Guillarme, 510 Systematic evaluation of matrix effects in supercritical fluid chromatography versus liquid 511 chromatography coupled to mass spectrometry for biological samples, Journal of 512 Chromatography B, 1079 (2018) 51-61.

[38] R. Hofstetter, G.M. Fassauer, A. Link, Supercritical fluid extraction (SFE) of ketamine
metabolites from dried urine and on-line quantification by supercritical fluid chromatography
and single mass detection (on-line SFE–SFC–MS), Journal of Chromatography B, 1076
(2018) 77-83.

[39] G.M. Fassauer, R. Hofstetter, M. Hasan, S. Oswald, C. Modess, W. Siegmund, A. Link,
Ketamine metabolites with antidepressant effects: Fast, economical, and eco-friendly
enantioselective separation based on supercritical-fluid chromatography (SFC) and single
quadrupole MS detection, J Pharmaceut Biomed, 146 (2017) 410-419.

[40] D. Wolrab, P. Frühauf, C. Gerner, Direct coupling of supercritical fluid chromatography
with tandem mass spectrometry for the analysis of amino acids and related compounds:
Comparing electrospray ionization and atmospheric pressure chemical ionization, Analytica
Chimica Acta, 981 (2017) 106-115.

[41] X. Li, Y. Gao, J. Liu, G. Zhang, T. Zhang, A rapid analysis of piroxicam in beagle plasma
applying evaporation-free liquid-liquid extraction by supercritical fluid chromatographytandem mass spectrometry, Journal of Chromatography B, 1100-1101 (2018) 93-99.

[42] Y. Tao, Z. Zheng, Y. Yu, J. Xu, X. Liu, X. Wu, F. Dong, Y. Zheng, Supercritical fluid
chromatography–tandem mass spectrometry-assisted methodology for rapid enantiomeric
analysis of fenbuconazole and its chiral metabolites in fruits, vegetables, cereals, and soil,
Food Chemistry, 241 (2018) 32-39.

[43] A. Haglind, M. Hedeland, T. Arvidsson, C.E. Pettersson, Major signal suppression from
metal ion clusters in SFC/ESI-MS - Cause and effects, Journal of Chromatography B, 1084
(2018) 96-105.

[44] L. Nováková, V. Desfontaine, F. Ponzetto, R. Nicoli, M. Saugy, J.-L. Veuthey, D.
Guillarme, Fast and sensitive supercritical fluid chromatography - tandem mass spectrometry
multi-class screening method for the determination of doping agents in urine, 2016.

[45] S. Hegstad, H. Havnen, A. Helland, O. Spigset, J. Frost, Enantiomeric separation and
quantification of R/S-amphetamine in urine by ultra-high performance supercritical fluid
chromatography tandem mass spectrometry, Journal of Chromatography B, 1077-1078
(2018) 7-12.

[46] L. Wang, J. Wang, J. Zhang, Q. Jiang, L. Zhao, T. Zhang, Simultaneous determination of
topiramate, carbamazepine, oxcarbazepine and its major metabolite in human plasma by
SFC-ESI-MS/MS with polarity switching: Application to therapeutic drug monitoring, Arabian
Journal of Chemistry, DOI https://doi.org/10.1016/j.arabjc.2016.09.016(2016).

[47] L. Herpin, E. Bichon, L. Rambaud, F. Monteau, B. Le Bizec, Comparison between liquid
chromatography and supercritical fluid chromatography coupled to mass spectrometry for
beta-agonists screening in feeding stuff, Journal of Chromatography B, 1086 (2018) 130-137.

[48] J. Teubel, B. Wüst, C.G. Schipke, O. Peters, M.K. Parr, Methods in endogenous steroid
profiling – A comparison of gas chromatography mass spectrometry (GC–MS) with
supercritical fluid chromatography tandem mass spectrometry (SFC-MS/MS), J. Chromatogr.
A, 1554 (2018) 101-116.

[49] H. Jambo, A. Dispas, H.T. Avohou, S. André, C. Hubert, P. Lebrun, É. Ziemons, P.
Hubert, Implementation of a generic SFC-MS method for the quality control of potentially
counterfeited medicinal cannabis with synthetic cannabinoids, Journal of Chromatography B,
1092 (2018) 332-342.

[50] V. Pilařová, T. Gottvald, P. Svoboda, O. Novák, K. Benešová, S. Běláková, L. Nováková,
Development and optimization of ultra-high performance supercritical fluid chromatography
mass spectrometry method for high-throughput determination of tocopherols and tocotrienols
in human serum, Analytica Chimica Acta, 934 (2016) 252-265.

[51] L. Nováková, M. Rentsch, A. Grand-Guillaume Perrenoud, R. Nicoli, M. Saugy, J.L.
Veuthey, D. Guillarme, Ultra high performance supercritical fluid chromatography coupled
with tandem mass spectrometry for screening of doping agents. II: Analysis of biological
samples, Analytica Chimica Acta, 853 (2015) 647-659.

565 [52] V. Desfontaine, L. Novakova, F. Ponzetto, R. Nicoli, M. Saugy, J.L. Veuthey, D. 566 Guillarme, Liquid chromatography and supercritical fluid chromatography as alternative 567 techniques to gas chromatography for the rapid screening of anabolic agents in urine, J 568 Chromatogr A, 1451 (2016) 145-155.

[53] C. Jenkinson, A. Taylor, K.-H. Storbeck, M. Hewison, Analysis of multiple vitamin D
metabolites by ultra-performance supercritical fluid chromatography-tandem mass
spectrometry (UPSFC-MS/MS), Journal of Chromatography B, 1087-1088 (2018) 43-48.

[54] V. Desfontaine, L. Nováková, F. Ponzetto, R. Nicoli, M. Saugy, J.-L. Veuthey, D.
Guillarme, Liquid chromatography and supercritical fluid chromatography as alternative
techniques to gas chromatography for the rapid screening of anabolic agents in urine, J.
Chromatogr. A, 1451 (2016) 145-155.

[55] V. Desfontaine, A. Tarafder, J. Hill, J. Fairchild, A. Grand-Guillaume Perrenoud, J.-L.
Veuthey, D. Guillarme, A systematic investigation of sample diluents in modern supercritical
fluid chromatography, J. Chromatogr. A, 1511 (2017) 122-131.

579 [56] X. Shi, W. Yang, S. Qiu, J. Hou, W. Wu, D. Guo, Systematic profiling and comparison of 580 the lipidomes from Panax ginseng, P. quinquefolius, and P. notoginseng by ultrahigh 581 performance supercritical fluid chromatography/high-resolution mass spectrometry and ion 582 mobility-derived collision cross section measurement, J. Chromatogr. A, 1548 (2018) 64-75.

[57] Z.-J. Jiang, X.-L. Cao, H. Li, C. Zhang, A.M. Abd El-Aty, F. Jin, H. Shao, M.-J. Jin, S.-S.
Wang, Y.-X. She, J. Wang, Fast determination of alkylphenol ethoxylates in leafy vegetables
using a modified quick, easy, cheap, effective, rugged, and safe method and ultra-high
performance supercritical fluid chromatography–tandem mass spectrometry, J. Chromatogr.
A, 1525 (2017) 161-172.

[58] S. Al Hamimi, M. Sandahl, M. Armeni, C. Turner, P. Spégel, Screening of stationary
phase selectivities for global lipid profiling by ultrahigh performance supercritical fluid
chromatography, J. Chromatogr. A, 1548 (2018) 76-82.

591 [59] J. Duval, C. Colas, P. Bonnet, E. Lesellier, Hyphenation of ultra-high performance 592 supercritical fluid chromatography with atmospheric pressure chemical ionisation high 593 resolution mass spectrometry: Part 2. Study of chromatographic and mass spectrometry

parameters for the analysis of natural non-polar compounds, J. Chromatogr. A, 1596 (2019)199-208.

[60] V. Pauk, T. Pluháček, V. Havlíček, K. Lemr, Ultra-high performance supercritical fluid
chromatography-mass spectrometry procedure for analysis of monosaccharides from plant
gum binders, Analytica Chimica Acta, 989 (2017) 112-120.

599 [61] Y. Huang, T. Zhang, H. Zhou, Y. Feng, C. Fan, W. Chen, J. Crommen, Z. Jiang, Fast 600 separation of triterpenoid saponins using supercritical fluid chromatography coupled with 601 single quadrupole mass spectrometry, J Pharmaceut Biomed, 121 (2016) 22-29.

[62] Y. Huang, Y. Feng, G. Tang, M. Li, T. Zhang, M. Fillet, J. Crommen, Z. Jiang,
Development and validation of a fast SFC method for the analysis of flavonoids in plant
extracts, J Pharmaceut Biomed, 140 (2017) 384-391.

[63] L.-F. Nothias, S. Boutet-Mercey, X. Cachet, E. De La Torre, L. Laboureur, J.-F. Gallard,
P. Retailleau, A. Brunelle, P.C. Dorrestein, J. Costa, L.M. Bedoya, F. Roussi, P. Leyssen, J.
Alcami, J. Paolini, M. Litaudon, D. Touboul, Environmentally Friendly Procedure Based on
Supercritical Fluid Chromatography and Tandem Mass Spectrometry Molecular Networking
for the Discovery of Potent Antiviral Compounds from Euphorbia semiperfoliata, Journal of
Natural Products, 80 (2017) 2620-2629.

[64] A. Grand-Guillaume Perrenoud, D. Guillarme, J. Boccard, J.-L. Veuthey, D. Barron, S.
Moco, Ultra-high performance supercritical fluid chromatography coupled with quadrupoletime-of-flight mass spectrometry as a performing tool for bioactive analysis, J. Chromatogr.
A, 1450 (2016) 101-111.

[65] I. González-Mariño, K.V. Thomas, M.J. Reid, Determination of cannabinoid and
synthetic cannabinoid metabolites in wastewater by liquid–liquid extraction and ultra-high
performance supercritical fluid chromatography-tandem mass spectrometry, Drug Testing
and Analysis, 10 (2018) 222-228.

[66] T. Toyo'oka, R. Kikura-Hanajiri, A Reliable Method for the Separation and Detection of
Synthetic Cannabinoids by Supercritical Fluid Chromatography with Mass Spectrometry, and
Its Application to Plant Products, Chemical and Pharmaceutical Bulletin, 63 (2015) 762-769.

[67] M. Wang, Y.-H. Wang, B. Avula, M.M. Radwan, A.S. Wanas, Z. Mehmedic, J. van
Antwerp, M.A. ElSohly, I.A. Khan, Quantitative Determination of Cannabinoids in Cannabis
and Cannabis Products Using Ultra-High-Performance Supercritical Fluid Chromatography
and Diode Array/Mass Spectrometric Detection, 62 (2017) 602-611.

[68] S. Breitenbach, W.F. Rowe, B. McCord, I.S. Lurie, Assessment of ultra high
performance supercritical fluid chromatography as a separation technique for the analysis of
seized drugs: Applicability to synthetic cannabinoids, J. Chromatogr. A, 1440 (2016) 201211.

[69] H. Segawa, Y. T. Iwata, T. Yamamuro, K. Kuwayama, K. Tsujikawa, T. Kanamori, H.
Inoue, Differentiation of ring-substituted regioisomers of amphetamine and
methamphetamine by supercritical fluid chromatography, Drug Testing and Analysis, 9
(2017) 389-398.

[70] H. Segawa, Y.T. Iwata, T. Yamamuro, K. Kuwayama, K. Tsujikawa, T. Kanamori, H.
Inoue, Enantioseparation of methamphetamine by supercritical fluid chromatography with
cellulose-based packed column, Forensic Science International, 273 (2017) 39-44.

[71] M.K. Parr, B. Wuest, E. Naegele, J.F. Joseph, M. Wenzel, A.H. Schmidt, M. Stanic, X.
de la Torre, F. Botrè, SFC-MS/MS as an orthogonal technique for improved screening of
polar analytes in anti-doping control, Analytical and Bioanalytical Chemistry, 408 (2016)
6789-6797.

[72] L. Nováková, A. Grand-Guillaume Perrenoud, R. Nicoli, M. Saugy, J.-L. Veuthey, D.
Guillarme, Ultra high performance supercritical fluid chromatography coupled with tandem
mass spectrometry for screening of doping agents. I: Investigation of mobile phase and MS
conditions, Analytica Chimica Acta, 853 (2015) 637-646.

[73] J.L. Quanson, M.A. Stander, E. Pretorius, C. Jenkinson, A.E. Taylor, K.-H. Storbeck,
High-throughput analysis of 19 endogenous androgenic steroids by ultra-performance
convergence chromatography tandem mass spectrometry, Journal of Chromatography B,
1031 (2016) 131-138.

[74] M. Doué, C. West, E. Bichon, B. Le Bizec, E. Lesellier, Supercritical fluid
chromatography applied to the highly selective isolation of urinary steroid hormones prior to
GC/MS analysis, Journal of Chromatography B, 1086 (2018) 97-104.

[75] L. Borovcova, V. Pauk, K. Lemr, Analysis of new psychoactive substances in human
urine by ultra-high performance supercritical fluid and liquid chromatography: Validation and
comparison, J Sep Sci, 41 (2018) 2288-2295.

[76] V. Desfontaine, G.L. Losacco, Y. Gagnebin, J. Pezzatti, W.P. Farrell, V. González-Ruiz,
S. Rudaz, J.-L. Veuthey, D. Guillarme, Applicability of supercritical fluid chromatography –
mass spectrometry to metabolomics. I – Optimization of separation conditions for the
simultaneous analysis of hydrophilic and lipophilic substances, J. Chromatogr. A, 1562
(2018) 96-107.

[77] R. Joyce, V. Kuziene, X. Zou, X. Wang, F. Pullen, R.L. Loo, Development and validation
of an ultra-performance liquid chromatography quadrupole time of flight mass spectrometry
method for rapid quantification of free amino acids in human urine, Amino Acids, 48 (2016)
219-234.

[78] Y. Huang, G. Tang, T. Zhang, M. Fillet, J. Crommen, Z. Jiang, Supercritical fluid
chromatography in traditional Chinese medicine analysis, J Pharmaceut Biomed, 147 (2018)
666 65-80.

[79] K. Taguchi, E. Fukusaki, T. Bamba, Simultaneous analysis for water- and fat-soluble
vitamins by a novel single chromatography technique unifying supercritical fluid
chromatography and liquid chromatography, J. Chromatogr. A, 1362 (2014) 270-277.

[80] S. Bieber, G. Greco, S. Grosse, T. Letzel, RPLC-HILIC and SFC with Mass
Spectrometry: Polarity-Extended Organic Molecule Screening in Environmental (Water)
Samples, Analytical Chemistry, 89 (2017) 7907-7914.

[81] V. Pilařová, K. Plachká, M.A. Khalikova, F. Svec, L. Nováková, Recent developments in
supercritical fluid chromatography – mass spectrometry: Is it a viable option for analysis of
complex samples?, TrAC Trends in Analytical Chemistry, DOI
https://doi.org/10.1016/j.trac.2018.12.023(2019).

[82] M. Zoccali, D. Giuffrida, F. Salafia, S.V. Giofrè, L. Mondello, Carotenoids and
apocarotenoids determination in intact human blood samples by online supercritical fluid
extraction-supercritical fluid chromatography-tandem mass spectrometry, Analytica Chimica
Acta, 1032 (2018) 40-47.

[83] A.P. Wicker, D.D. Carlton, K. Tanaka, M. Nishimura, V. Chen, T. Ogura, W. Hedgepeth,
K.A. Schug, On-line supercritical fluid extraction—supercritical fluid chromatography-mass
spectrometry of polycyclic aromatic hydrocarbons in soil, Journal of Chromatography B, 1086
(2018) 82-88.

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687 Figure captions

Figure 1: Representations of the five most common SFC–MS interfaces. (A) "direct coupling" interface, (B) "pre-UV and BPR splitter without sheath pump" interface, (C) "pressure control fluid" interface, (D) "pre-BPR splitter with sheath pump" interface, (E) "BPR and sheath pump with no splitter" interface. Reprinted from J. Chromatogr. B, Vol. 1083; D. Guillarme, V. Desfontaine, S. Heinisch, J.-L. Veuthey; What are the current solutions for interfacing supercritical fluid chromatography and mass spectrometry?, pp 160-170 [ref 25]. Copyright 2018, with permission from Elsevier.

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Figure 2: A comparison of sensitivity between two different triple quadrupole platforms, i.e., 696 Modern MS/MS device, namely Waters Xevo TQ-S (A) and old-generation MS/MS device, 697 namely Waters TQD (B) in UHPSFC-MS/MS and UHPLC-MS/MS modes. Data used for this 698 699 comparison were taken from [62]. Reprinted from Anal. Chim. Acta, Vol. 853; L. Nováková, 700 M. Rentsch, A. Grand-Guillaume Perrenoud, R. Nicoli, M. Saugy, J.L. Veuthey, D. Guillarme; 701 Ultra high perfomance supercritical fluid chromatography coupled with tandem mass spectrometry for screening of doping agents II: Analysis of biological samples; pp 647-659 702 703 [ref 50]. Copyright 2015, with permission from Elsevier.

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705 Figure 3: Example of chromatograms (UHPSFC-QqToF-MS traces) obtained on Diol column, highlighting chemically diverse compounds with Log P, H-bond capability and 706 molecular mass respectively: carotenoid zeaxantin (10.92, 2, 568.43 Da); alkaloid sparteine 707 (2.84, 2, 234.21 Da); triterpenoid lupeol (10.46, 2, 426.39 Da); the iridoid gentiopicroside 708 (-3.03, 13, 356.11 Da); saponin ginsenoside-Rd (3.38, 30, 946.55 Da); diterpenoid paclitaxel 709 (3.95, 19, 853.33 Da). Reprinted from J. Chromatogr. A, Vol. 1450; A. Grand-Guillaume 710 711 Perrenoud, D. Guillarme, J. Boccard, J.-L. Veuthey, D. Barron, S. Moco; Ultra-high 712 performance supercritical fluid chromatography coupled with guadrupole-time-of-flight mass

spectrometry as a performing tool for bioactive analysis; pp 101-111 [ref 60]. Copyright 2016,
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Figure 4: Chromatograms of nine steroids and related metabolites for injection of urine
spiked at 10 ng/mL in UHPSFC-MS/MS.). Reprinted from *J. Chromatogr. A*, Vol. *1451*; V.
Desfontaine, L. Novakova, F. Ponzetto, R. Nicoli, M. Saugy, J.L. Veuthey, D. Guillarme;
Liquid chromatography and supercritical fluid chromatography as alternative techniques to
gas chromatography for the rapid screening of anabolic agents in urine; pp 145-155 [ref 51].
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722

Figure 5: Chromatogram obtained for the simultaneous injection of tricosanoic acid 723 724 and raffinose, using acetonitrile/water (50:50)as sample diluent and unified chromatography gradient conditions. Reprinted from J. Chromatogr. A, Vol. 1562; V. 725 Desfontaine, G.L. Losacco, Y. Gagnebin, J. Pezzatti, W.P. Farrell, V. González-Ruiz, S. 726 727 Rudaz, J.-L. Veuthey, D. Guillarme; Applicability of supercritical fluid chromatography – mass 728 spectrometry to metabolomics. I – Optimization of separation conditions for the simultaneous 729 analysis of hydrophilic and lipophilic substance; pp 96-107 [ref 72]. Copyright 2018, with 730 permission from Elsevier.















