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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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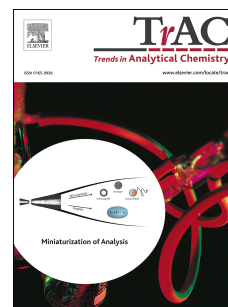
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1 **SUPERCritical FLUID CHROMATOGRAPHY – MASS**
2 **SPECTROMETRY: RECENT EVOLUTION AND CURRENT TRENDS**

3
4 **AUTHORS:** Gioacchino Luca Losacco¹, Jean-Luc Veuthey¹, Davy Guillarme^{1*}

5
6 (1) School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CMU-
7 Rue Michel Servet 1, 1211 Geneva 4, Switzerland

8 *corresponding author

9
10 **CORRESPONDENCE:**

11 School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CMU -
12 Rue Michel-Servet 1, 1211 Geneva 4, Switzerland

13 Phone: +41 22 379 34 63

14 E-mail: Davy.guillarme@unige.ch

15
16 **KEYWORDS:**

17 Supercritical fluid chromatography; interfaces; matrix effects; sensitivity; doping agents;
18 metabolomics

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23 **HIGHLIGHTS:**

- 24 • A detailed summary on SFC-MS interfaces is given, with emphasis on current issues
25 and potential solutions.
- 26 • Differences between LC-MS and SFC-MS in terms of matrix effects generated are
27 highlighted.
- 28 • Sensitivity under SFC-MS has been demonstrated to be comparable to what it can be
29 reached in LC-MS conditions.
- 30 • Applications for SFC-MS are shifting towards the analysis of compounds with
31 increasing polarity and analytes available in complex matrices.
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46 **ABSTRACT**

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

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70 1. Introduction

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

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

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

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

118 **2. UHPSFC-MS interfaces**

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

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

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

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

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

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

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

177 especially since it might lead to more severe issues such as band broadening or even loss of
178 the chromatographic separation.

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

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

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

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

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

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

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

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

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

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

302 An important field of application is the analysis of natural products. Indeed, there have
303 already been developments and successful implementations in the past, however, now the
304 constantly growing use of high resolutions MS instruments (HRMS), hyphenated not only to
305 LC but also to SFC, has pushed the latter even further in this area. Besides the analysis of
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
309 under SFC-MS, such as plant metabolites with interesting potential as drugs (Fig. 3) [63, 64].
310 A specific category, which also attracts attention, is cannabinoids; indeed, the use of this

311 class of compounds is rapidly increasing, in both medical and forensic applications [65-67].
312 Today, SFC-MS can be considered as a complementary technique to LC-MS, with an
313 interesting ability in obtaining resolution of positional isomers and diastereomers, with a high
314 degree of orthogonality to LC [68]. Moreover, the methods developed in SFC-MS also fit well
315 with quality control requirements of real-life cannabis samples analysis [49], thanks to an
316 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
318 SFC-MS in the forensic and anti-doping control analysis. Indeed, there has been an
319 important number of papers recently released and focusing on several classes of
320 compounds: amphetamines [45, 69, 70], stimulants and sympathomimetic drugs [51, 71, 72]
321 or anabolic agents and steroids [44, 54, 73, 74] (Fig. 4). Researchers involved in the field of
322 anti-doping analysis are now testing new analytical techniques (such as SFC-MS), to find
323 possible advantages to the current state of the art represented by LC-MS. Furthermore, SFC
324 is not only being used as an analytical method but also employed in the sample preparation
325 stage [74], with the aim to replace older methods employed in the sample treatment.
326 Obviously, SFC-MS methods that wish to be employed in anti-doping laboratories also have
327 to be validated. This aspect is being currently investigated by several authors, with a growing
328 number of publications [38, 39, 45, 47, 49, 75] showing that the validation procedure in SFC-
329 MS yields similar, if not even better results than LC-MS. Indeed, during different validation
330 processes of SFC-MS methods, it was found that SFC-MS manages to provide better results
331 in terms of identification, reproducibility, precision and accuracy when compared to LC-MS
332 [47, 75]. These findings are extremely important in establishing SFC-MS itself as a technique
333 that is compatible with regulated bioanalytical laboratories.

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

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

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

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

366 **6. Conclusions**

367 The hyphenation of SFC to MS has undoubtedly known an impressive growth in the last five
368 years. The development of several SFC-MS geometry interfaces has enabled to couple both
369 systems, as well as a discrete handling of the supercritical fluid once the mobile phase is not
370 under the influence of the APBR module. There are, however, still aspects that necessitate to
371 be thoroughly covered to understand the influence of the CO₂ decompression and how to
372 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.

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

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

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

393

ACCEPTED MANUSCRIPT

394 **7. References**

- 395 [1] T. Kind, H. Tsugawa, T. Cajka, Y. Ma, Z.J. Lai, S.S. Mehta, G. Wohlgemuth, D.K.
396 Barupal, M.R. Showalter, M. Arita, O. Fiehn, Identification of small molecules using accurate
397 mass MS/MS search, *Mass Spectrometry Reviews*, 37 (2018) 513-532.
- 398 [2] A.H. Zhang, H. Sun, X.J. Wang, Mass spectrometry-driven drug discovery for
399 development of herbal medicine, *Mass Spectrometry Reviews*, 37 (2018) 307-320.
- 400 [3] N. de Kock, S.R. Acharya, S.J.K.A. Ubhayasekera, J. Bergquist, A Novel Targeted
401 Analysis of Peripheral Steroids by Ultra-Performance Supercritical Fluid Chromatography
402 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. Oganessian, 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 -guadecitabine in human plasma, whole blood and urine, *Journal of Chromatography B*, DOI
411 <https://doi.org/10.1016/j.jchromb.2019.01.011>(2019).
- 412 [6] O. Ciclet, D. Barron, S. Bajic, J.-L. Veuthey, D. Guillarme, A. Grand-Guillaume Perrenoud,
413 Natural compounds analysis using liquid and supercritical fluid chromatography hyphenated
414 to mass spectrometry: Evaluation of a new design of atmospheric pressure ionization source,
415 *Journal of Chromatography B*, 1083 (2018) 1-11.
- 416 [7] G. Hermann, M. Schwaiger, P. Volejnik, G. Koellensperger, ¹³C-labelled yeast as internal
417 standard for LC-MS/MS and LC high resolution MS based amino acid quantification in
418 human plasma, *J Pharmaceut Biomed*, 155 (2018) 329-334.
- 419 [8] B. Petrie, M.D. Camacho Muñoz, J. Martín, Stereoselective LC-MS/MS methodologies for
420 environmental analysis of chiral pesticides, *TrAC Trends in Analytical Chemistry*, 110 (2019)
421 249-258.

- 422 [9] C. Marchioni, I.D. de Souza, V.R. Acquaro, J.A. de Souza Crippa, V. Tumas, M.E.C.
423 Queiroz, Recent advances in LC-MS/MS methods to determine endocannabinoids in
424 biological samples: Application in neurodegenerative diseases, *Analytica Chimica Acta*, 1044
425 (2018) 12-28.
- 426 [10] W. Lv, X. Shi, S. Wang, G. Xu, Multidimensional liquid chromatography-mass
427 spectrometry for metabolomic and lipidomic analyses, *TrAC Trends in Analytical Chemistry*,
428 DOI <https://doi.org/10.1016/j.trac.2018.11.001>(2018).
- 429 [11] T. Higashi, M. Akaishi, M. Yokota, T. Suzuki, S. Ogawa, Y. Sugiura, T. Nishikawa, K.
430 Nishimoto, M. Suematsu, A method for determination of aldosterone in adrenal tributary
431 venous serum by derivatization using Girard P reagent isotopologues followed by LC/ESI-
432 MS/MS, *Journal of Chromatography B*, 1092 (2018) 106-113.
- 433 [12] A.-S. Claeson, S. Gouveia-Figueira, H. Stenlund, A.I. Johansson, A standardized
434 protocol for comparable analysis of GSH/GSSG by UHPLC-ESI-MS/MS for human plasma,
435 *Journal of Chromatography B*, 1104 (2019) 67-72.
- 436 [13] K. Hu, Y. Li, R. Ding, Y. Zhai, L. Chen, W. Qian, J. Yang, A simple, sensitive, and high-
437 throughput LC-APCI-MS/MS method for simultaneous determination of vitamin K1, vitamin
438 K1 2,3-epoxide in human plasma and its application to a clinical pharmacodynamic study of
439 warfarin, *Journal of Pharmaceutical and Biomedical Analysis*, 159 (2018) 82-91.
- 440 [14] S. Biswas, R. Mondal, A. Mukherjee, M. Sarkar, R.K. Kole, Simultaneous determination
441 and risk assessment of fipronil and its metabolites in sugarcane, using GC-ECD and
442 confirmation by GC-MS/MS, *Food Chemistry*, 272 (2019) 559-567.
- 443 [15] P. Fang, J.-Z. Pan, Q. Fang, A robust and extendable sheath flow interface with minimal
444 dead volume for coupling CE with ESI-MS, *Talanta*, 180 (2018) 376-382.
- 445 [16] S. Yin, Y. Yang, L. Wu, Y. Li, C. Sun, Recent advances in sample preparation and
446 analysis methods for vitamin D and its analogues in different matrices, *TrAC Trends in*
447 *Analytical Chemistry*, 110 (2019) 204-220.
- 448 [17] X. Zhang, X. Ding, J. Wang, B. Dean, Supercritical fluid chromatography-tandem mass
449 spectrometry for high throughput bioanalysis of small molecules in drug discovery, *J*
450 *Pharmaceut Biomed*, 164 (2019) 62-69.

- 451 [18] D.P. Poe, Chapter 2 - Theory of Supercritical Fluid Chromatography, in: C.F. Poole (Ed.)
452 Supercritical Fluid Chromatography, Elsevier2017, pp. 23-55.
- 453 [19] K.L. Williams, L.C. Sander, Enantiomer separations on chiral stationary phases in
454 supercritical fluid chromatography, *J. Chromatogr. A*, 785 (1997) 149-158.
- 455 [20] C. Berger, M. Perrut, Preparative supercritical fluid chromatography, *J. Chromatogr. A*,
456 505 (1990) 37-43.
- 457 [21] T.A. Berger, Chapter 7 - Evolution of Instrumentation for Analytical Scale Supercritical
458 Fluid Chromatography**This chapter draws upon large sections of TA Berger,
459 "Instrumentation for supercritical fluid chromatography," *J. Chromatogr. A*, 1421 (November
460 2015), 171–183. Reproduced with permission from Elsevier. ©Elsevier 2015, in: C.F. Poole
461 (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.
- 465 [23] U. Jumhawan, T. Bamba, Chapter 16 - Supercritical Fluid Chromatography, in: F. Pena-
466 Pereira, M. Tobiszewski (Eds.) *The Application of Green Solvents in Separation Processes*,
467 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.
- 470 [25] D. Guillarme, V. Desfontaine, S. Heinisch, J.-L. Veuthey, What are the current solutions
471 for interfacing supercritical fluid chromatography and mass spectrometry?, *Journal of*
472 *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.
- 476 [27] L. Akbal, G. Hopfgartner, Effects of liquid post-column addition in electrospray ionization
477 performance in supercritical fluid chromatography–mass spectrometry, *J. Chromatogr. A*,
478 1517 (2017) 176-184.

- 479 [28] M.K. Parr, B. Wüst, J. Teubel, J.F. Joseph, Splitless hyphenation of SFC with MS by
480 APCI, APPI, and ESI exemplified by steroids as model compounds, *Journal of*
481 *Chromatography B*, 1091 (2018) 67-78.
- 482 [29] Y. Fujito, Y. Hayakawa, Y. Izumi, T. Bamba, Importance of optimizing chromatographic
483 conditions and mass spectrometric parameters for supercritical fluid chromatography/mass
484 spectrometry, *J. Chromatogr. A*, 1508 (2017) 138-147.
- 485 [30] J. Duval, C. Colas, V. Pecher, M. Poujol, J.-F. Tranchant, E. Lesellier, Hyphenation of
486 ultra high performance supercritical fluid chromatography with atmospheric pressure
487 chemical ionisation high resolution mass spectrometry: Part 1. Study of the coupling
488 parameters for the analysis of natural non-polar compounds, *J. Chromatogr. A*, 1509 (2017)
489 132-140.
- 490 [31] F. Petruzzello, A. Grand-Guillaume Perrenoud, A. Thorimbert, M. Fogwill, S. Rezzi,
491 Quantitative Profiling of Endogenous Fat-Soluble Vitamins and Carotenoids in Human
492 Plasma Using an Improved UHPSFC-ESI-MS Interface, *Analytical Chemistry*, 89 (2017)
493 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.
- 499 [34] S. Wang, P. Qi, S. Di, J. Wang, S. Wu, X. Wang, Z. Wang, Q. Wang, X. Wang, C. Zhao,
500 Q. Li, Significant role of supercritical fluid chromatography - mass spectrometry in improving
501 the matrix effect and analytical efficiency during multi-pesticides residue analysis of complex
502 chrysanthemum samples, *Analytica Chimica Acta*, 1074 (2019) 108-116.
- 503 [35] P.J. Rudzki, E. Gniazdowska, K. Buś-Kwaśnik, Quantitative evaluation of the matrix
504 effect in bioanalytical methods based on LC-MS: A comparison of two approaches, *J*
505 *Pharmaceut Biomed*, 155 (2018) 314-319.

- 506 [36] A. Svan, M. Hedeland, T. Arvidsson, C.E. Pettersson, The differences in matrix effect
507 between supercritical fluid chromatography and reversed phase liquid chromatography
508 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.
- 513 [38] R. Hofstetter, G.M. Fassauer, A. Link, Supercritical fluid extraction (SFE) of ketamine
514 metabolites from dried urine and on-line quantification by supercritical fluid chromatography
515 and single mass detection (on-line SFE–SFC–MS), *Journal of Chromatography B*, 1076
516 (2018) 77-83.
- 517 [39] G.M. Fassauer, R. Hofstetter, M. Hasan, S. Oswald, C. Modess, W. Siegmund, A. Link,
518 Ketamine metabolites with antidepressant effects: Fast, economical, and eco-friendly
519 enantioselective separation based on supercritical-fluid chromatography (SFC) and single
520 quadrupole MS detection, *J Pharmaceut Biomed*, 146 (2017) 410-419.
- 521 [40] D. Wolrab, P. Frühauf, C. Gerner, Direct coupling of supercritical fluid chromatography
522 with tandem mass spectrometry for the analysis of amino acids and related compounds:
523 Comparing electrospray ionization and atmospheric pressure chemical ionization, *Analytica*
524 *Chimica Acta*, 981 (2017) 106-115.
- 525 [41] X. Li, Y. Gao, J. Liu, G. Zhang, T. Zhang, A rapid analysis of piroxicam in beagle plasma
526 applying evaporation-free liquid-liquid extraction by supercritical fluid chromatography-
527 tandem mass spectrometry, *Journal of Chromatography B*, 1100-1101 (2018) 93-99.
- 528 [42] Y. Tao, Z. Zheng, Y. Yu, J. Xu, X. Liu, X. Wu, F. Dong, Y. Zheng, Supercritical fluid
529 chromatography–tandem mass spectrometry-assisted methodology for rapid enantiomeric
530 analysis of fenbuconazole and its chiral metabolites in fruits, vegetables, cereals, and soil,
531 *Food Chemistry*, 241 (2018) 32-39.
- 532 [43] A. Haglind, M. Hedeland, T. Arvidsson, C.E. Pettersson, Major signal suppression from
533 metal ion clusters in SFC/ESI-MS - Cause and effects, *Journal of Chromatography B*, 1084
534 (2018) 96-105.

- 535 [44] L. Nováková, V. Desfontaine, F. Ponzetto, R. Nicoli, M. Saugy, J.-L. Veuthey, D.
536 Guillaume, Fast and sensitive supercritical fluid chromatography - tandem mass spectrometry
537 multi-class screening method for the determination of doping agents in urine, 2016.
- 538 [45] S. Hegstad, H. Havnen, A. Helland, O. Spigset, J. Frost, Enantiomeric separation and
539 quantification of R/S-amphetamine in urine by ultra-high performance supercritical fluid
540 chromatography tandem mass spectrometry, *Journal of Chromatography B*, 1077-1078
541 (2018) 7-12.
- 542 [46] L. Wang, J. Wang, J. Zhang, Q. Jiang, L. Zhao, T. Zhang, Simultaneous determination of
543 topiramate, carbamazepine, oxcarbazepine and its major metabolite in human plasma by
544 SFC-ESI-MS/MS with polarity switching: Application to therapeutic drug monitoring, *Arabian*
545 *Journal of Chemistry*, DOI <https://doi.org/10.1016/j.arabjc.2016.09.016>(2016).
- 546 [47] L. Herpin, E. Bichon, L. Rambaud, F. Monteau, B. Le Bizec, Comparison between liquid
547 chromatography and supercritical fluid chromatography coupled to mass spectrometry for
548 beta-agonists screening in feeding stuff, *Journal of Chromatography B*, 1086 (2018) 130-137.
- 549 [48] J. Teubel, B. Wüst, C.G. Schipke, O. Peters, M.K. Parr, Methods in endogenous steroid
550 profiling – A comparison of gas chromatography mass spectrometry (GC–MS) with
551 supercritical fluid chromatography tandem mass spectrometry (SFC-MS/MS), *J. Chromatogr.*
552 *A*, 1554 (2018) 101-116.
- 553 [49] H. Jambo, A. Dispas, H.T. Avohou, S. André, C. Hubert, P. Lebrun, É. Ziemons, P.
554 Hubert, Implementation of a generic SFC-MS method for the quality control of potentially
555 counterfeited medicinal cannabis with synthetic cannabinoids, *Journal of Chromatography B*,
556 1092 (2018) 332-342.
- 557 [50] V. Pilařová, T. Gottvald, P. Svoboda, O. Novák, K. Benešová, S. Běláková, L. Nováková,
558 Development and optimization of ultra-high performance supercritical fluid chromatography
559 mass spectrometry method for high-throughput determination of tocopherols and tocotrienols
560 in human serum, *Analytica Chimica Acta*, 934 (2016) 252-265.
- 561 [51] L. Nováková, M. Rentsch, A. Grand-Guillaume Perrenoud, R. Nicoli, M. Saugy, J.L.
562 Veuthey, D. Guillaume, Ultra high performance supercritical fluid chromatography coupled
563 with tandem mass spectrometry for screening of doping agents. II: Analysis of biological
564 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 Guillaume, 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.
- 569 [53] C. Jenkinson, A. Taylor, K.-H. Storbeck, M. Hewison, Analysis of multiple vitamin D
570 metabolites by ultra-performance supercritical fluid chromatography-tandem mass
571 spectrometry (UPSFC-MS/MS), Journal of Chromatography B, 1087-1088 (2018) 43-48.
- 572 [54] V. Desfontaine, L. Nováková, F. Ponzetto, R. Nicoli, M. Saugy, J.-L. Veuthey, D.
573 Guillaume, Liquid chromatography and supercritical fluid chromatography as alternative
574 techniques to gas chromatography for the rapid screening of anabolic agents in urine, J.
575 Chromatogr. A, 1451 (2016) 145-155.
- 576 [55] V. Desfontaine, A. Tarafder, J. Hill, J. Fairchild, A. Grand-Guillaume Perrenoud, J.-L.
577 Veuthey, D. Guillaume, A systematic investigation of sample diluents in modern supercritical
578 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.
- 583 [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.
584 Wang, Y.-X. She, J. Wang, Fast determination of alkylphenol ethoxylates in leafy vegetables
585 using a modified quick, easy, cheap, effective, rugged, and safe method and ultra-high
586 performance supercritical fluid chromatography–tandem mass spectrometry, J. Chromatogr.
587 A, 1525 (2017) 161-172.
- 588 [58] S. Al Hamimi, M. Sandahl, M. Armeni, C. Turner, P. Spéjel, Screening of stationary
589 phase selectivities for global lipid profiling by ultrahigh performance supercritical fluid
590 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

- 594 parameters for the analysis of natural non-polar compounds, *J. Chromatogr. A*, 1596 (2019)
595 199-208.
- 596 [60] V. Pauk, T. Pluháček, V. Havlíček, K. Lemr, Ultra-high performance supercritical fluid
597 chromatography-mass spectrometry procedure for analysis of monosaccharides from plant
598 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.
- 602 [62] Y. Huang, Y. Feng, G. Tang, M. Li, T. Zhang, M. Fillet, J. Crommen, Z. Jiang,
603 Development and validation of a fast SFC method for the analysis of flavonoids in plant
604 extracts, *J Pharmaceut Biomed*, 140 (2017) 384-391.
- 605 [63] L.-F. Nothias, S. Boutet-Mercey, X. Cachet, E. De La Torre, L. Laboureur, J.-F. Gallard,
606 P. Retailleau, A. Brunelle, P.C. Dorrestein, J. Costa, L.M. Bedoya, F. Roussi, P. Leysen, J.
607 Alcamí, J. Paolini, M. Litaudon, D. Touboul, Environmentally Friendly Procedure Based on
608 Supercritical Fluid Chromatography and Tandem Mass Spectrometry Molecular Networking
609 for the Discovery of Potent Antiviral Compounds from *Euphorbia semiperfoliata*, *Journal of*
610 *Natural Products*, 80 (2017) 2620-2629.
- 611 [64] A. Grand-Guillaume Perrenoud, D. Guillarme, J. Boccard, J.-L. Veuthey, D. Barron, S.
612 Moco, Ultra-high performance supercritical fluid chromatography coupled with quadrupole-
613 time-of-flight mass spectrometry as a performing tool for bioactive analysis, *J. Chromatogr.*
614 *A*, 1450 (2016) 101-111.
- 615 [65] I. González-Mariño, K.V. Thomas, M.J. Reid, Determination of cannabinoid and
616 synthetic cannabinoid metabolites in wastewater by liquid-liquid extraction and ultra-high
617 performance supercritical fluid chromatography-tandem mass spectrometry, *Drug Testing*
618 *and Analysis*, 10 (2018) 222-228.
- 619 [66] T. Toyo'oka, R. Kikura-Hanajiri, A Reliable Method for the Separation and Detection of
620 Synthetic Cannabinoids by Supercritical Fluid Chromatography with Mass Spectrometry, and
621 Its Application to Plant Products, *Chemical and Pharmaceutical Bulletin*, 63 (2015) 762-769.

- 622 [67] M. Wang, Y.-H. Wang, B. Avula, M.M. Radwan, A.S. Wanas, Z. Mehmedic, J. van
623 Antwerp, M.A. ElSohly, I.A. Khan, Quantitative Determination of Cannabinoids in Cannabis
624 and Cannabis Products Using Ultra-High-Performance Supercritical Fluid Chromatography
625 and Diode Array/Mass Spectrometric Detection, 62 (2017) 602-611.
- 626 [68] S. Breitenbach, W.F. Rowe, B. McCord, I.S. Lurie, Assessment of ultra high
627 performance supercritical fluid chromatography as a separation technique for the analysis of
628 seized drugs: Applicability to synthetic cannabinoids, J. Chromatogr. A, 1440 (2016) 201-
629 211.
- 630 [69] H. Segawa, Y. T. Iwata, T. Yamamuro, K. Kuwayama, K. Tsujikawa, T. Kanamori, H.
631 Inoue, Differentiation of ring-substituted regioisomers of amphetamine and
632 methamphetamine by supercritical fluid chromatography, Drug Testing and Analysis, 9
633 (2017) 389-398.
- 634 [70] H. Segawa, Y.T. Iwata, T. Yamamuro, K. Kuwayama, K. Tsujikawa, T. Kanamori, H.
635 Inoue, Enantioseparation of methamphetamine by supercritical fluid chromatography with
636 cellulose-based packed column, Forensic Science International, 273 (2017) 39-44.
- 637 [71] M.K. Parr, B. Wuest, E. Naegele, J.F. Joseph, M. Wenzel, A.H. Schmidt, M. Stanic, X.
638 de la Torre, F. Botrè, SFC-MS/MS as an orthogonal technique for improved screening of
639 polar analytes in anti-doping control, Analytical and Bioanalytical Chemistry, 408 (2016)
640 6789-6797.
- 641 [72] L. Nováková, A. Grand-Guillaume Perrenoud, R. Nicoli, M. Saugy, J.-L. Veuthey, D.
642 Guillaume, Ultra high performance supercritical fluid chromatography coupled with tandem
643 mass spectrometry for screening of doping agents. I: Investigation of mobile phase and MS
644 conditions, Analytica Chimica Acta, 853 (2015) 637-646.
- 645 [73] J.L. Quanson, M.A. Stander, E. Pretorius, C. Jenkinson, A.E. Taylor, K.-H. Storbeck,
646 High-throughput analysis of 19 endogenous androgenic steroids by ultra-performance
647 convergence chromatography tandem mass spectrometry, Journal of Chromatography B,
648 1031 (2016) 131-138.
- 649 [74] M. Doué, C. West, E. Bichon, B. Le Bizec, E. Lesellier, Supercritical fluid
650 chromatography applied to the highly selective isolation of urinary steroid hormones prior to
651 GC/MS analysis, Journal of Chromatography B, 1086 (2018) 97-104.

- 652 [75] L. Borovcova, V. Pauk, K. Lemr, Analysis of new psychoactive substances in human
653 urine by ultra-high performance supercritical fluid and liquid chromatography: Validation and
654 comparison, *J Sep Sci*, 41 (2018) 2288-2295.
- 655 [76] V. Desfontaine, G.L. Losacco, Y. Gagnebin, J. Pezzatti, W.P. Farrell, V. González-Ruiz,
656 S. Rudaz, J.-L. Veuthey, D. Guillarme, Applicability of supercritical fluid chromatography –
657 mass spectrometry to metabolomics. I – Optimization of separation conditions for the
658 simultaneous analysis of hydrophilic and lipophilic substances, *J. Chromatogr. A*, 1562
659 (2018) 96-107.
- 660 [77] R. Joyce, V. Kuziene, X. Zou, X. Wang, F. Pullen, R.L. Loo, Development and validation
661 of an ultra-performance liquid chromatography quadrupole time of flight mass spectrometry
662 method for rapid quantification of free amino acids in human urine, *Amino Acids*, 48 (2016)
663 219-234.
- 664 [78] Y. Huang, G. Tang, T. Zhang, M. Fillet, J. Crommen, Z. Jiang, Supercritical fluid
665 chromatography in traditional Chinese medicine analysis, *J Pharmaceut Biomed*, 147 (2018)
666 65-80.
- 667 [79] K. Taguchi, E. Fukusaki, T. Bamba, Simultaneous analysis for water- and fat-soluble
668 vitamins by a novel single chromatography technique unifying supercritical fluid
669 chromatography and liquid chromatography, *J. Chromatogr. A*, 1362 (2014) 270-277.
- 670 [80] S. Bieber, G. Greco, S. Grosse, T. Letzel, RPLC-HILIC and SFC with Mass
671 Spectrometry: Polarity-Extended Organic Molecule Screening in Environmental (Water)
672 Samples, *Analytical Chemistry*, 89 (2017) 7907-7914.
- 673 [81] V. Pilařová, K. Plachká, M.A. Khalikova, F. Svec, L. Nováková, Recent developments in
674 supercritical fluid chromatography – mass spectrometry: Is it a viable option for analysis of
675 complex samples?, *TrAC Trends in Analytical Chemistry*, DOI
676 <https://doi.org/10.1016/j.trac.2018.12.023>(2019).
- 677 [82] M. Zoccali, D. Giuffrida, F. Salafia, S.V. Giofrè, L. Mondello, Carotenoids and
678 apocarotenoids determination in intact human blood samples by online supercritical fluid
679 extraction-supercritical fluid chromatography-tandem mass spectrometry, *Analytica Chimica*
680 *Acta*, 1032 (2018) 40-47.

681 [83] A.P. Wicker, D.D. Carlton, K. Tanaka, M. Nishimura, V. Chen, T. Ogura, W. Hedgepeth,
682 K.A. Schug, On-line supercritical fluid extraction—supercritical fluid chromatography-mass
683 spectrometry of polycyclic aromatic hydrocarbons in soil, *Journal of Chromatography B*, 1086
684 (2018) 82-88.

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687 **Figure captions**

688 Figure 1: Representations of the five most common SFC–MS interfaces. (A) “*direct coupling*”
689 interface, (B) “*pre-UV and BPR splitter without sheath pump*” interface, (C) “*pressure control*
690 *fluid*” interface, (D) “*pre-BPR splitter with sheath pump*” interface, (E) “*BPR and sheath pump*
691 *with no splitter*” interface. Reprinted from *J. Chromatogr. B*, Vol. 1083; D. Guillarme, V.
692 Desfontaine, S. Heinisch, J.-L. Veuthey; What are the current solutions for interfacing
693 supercritical fluid chromatography and mass spectrometry?, pp 160-170 [ref 25]. Copyright
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695

696 Figure 2: A comparison of sensitivity between two different triple quadrupole platforms, i.e.,
697 Modern MS/MS device, namely Waters Xevo TQ-S (A) and old-generation MS/MS device,
698 namely Waters TQD (B) in UHPSFC–MS/MS and UHPLC–MS/MS modes. Data used for this
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 performance supercritical fluid chromatography coupled with tandem mass
702 spectrometry for screening of doping agents II: Analysis of biological samples; pp 647-659
703 [ref 50]. Copyright 2015, with permission from Elsevier.

704

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

713 spectrometry as a performing tool for bioactive analysis; pp 101-111 [ref 60]. Copyright 2016,
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715

716 Figure 4: Chromatograms of nine steroids and related metabolites for injection of urine
717 spiked at 10 ng/mL in UHPSFC-MS/MS.). Reprinted from *J. Chromatogr. A*, Vol. 1451; V.
718 Desfontaine, L. Novakova, F. Ponzetto, R. Nicoli, M. Saugy, J.L. Veuthey, D. Guillarme;
719 Liquid chromatography and supercritical fluid chromatography as alternative techniques to
720 gas chromatography for the rapid screening of anabolic agents in urine; pp 145-155 [ref 51].
721 Copyright 2016, with permission from Elsevier.

722

723 Figure 5: Chromatogram obtained for the simultaneous injection of tricosanoic acid
724 and raffinose, using acetonitrile/water (50:50) as sample diluent and
725 unified chromatography gradient conditions. Reprinted from *J. Chromatogr. A*, Vol. 1562; V.
726 Desfontaine, G.L. Losacco, Y. Gagnebin, J. Pezzatti, W.P. Farrell, V. González-Ruiz, S.
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
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Figure 1

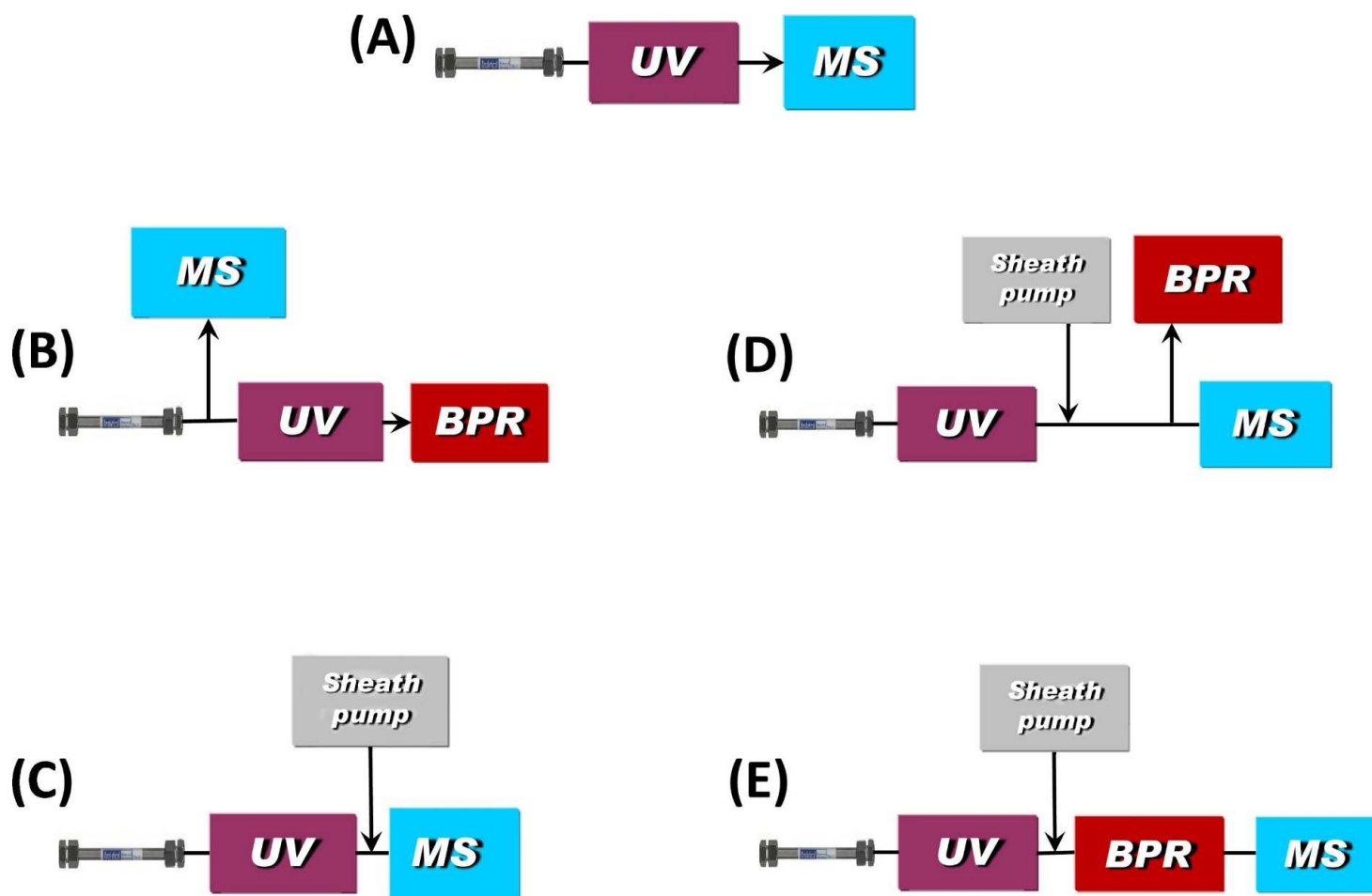


Figure 2

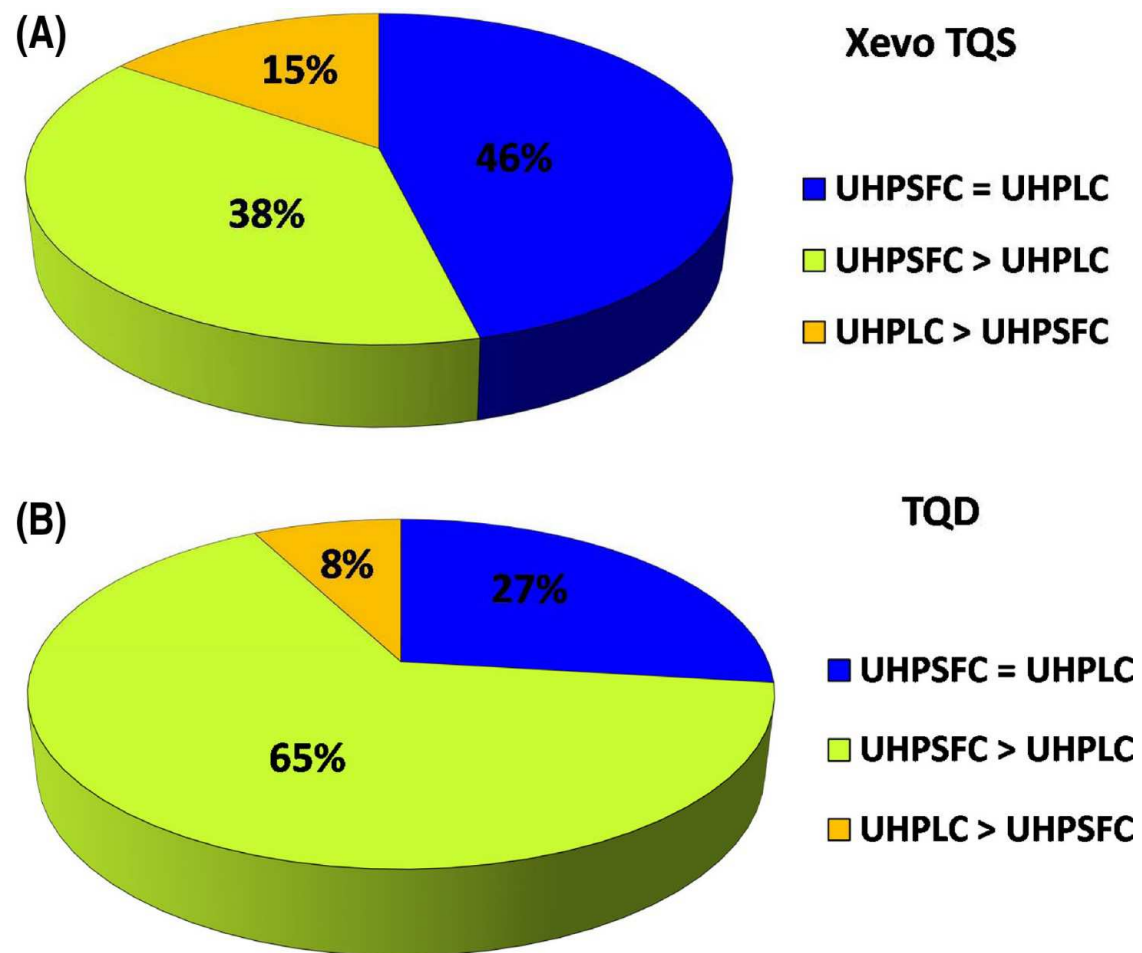


Figure 4

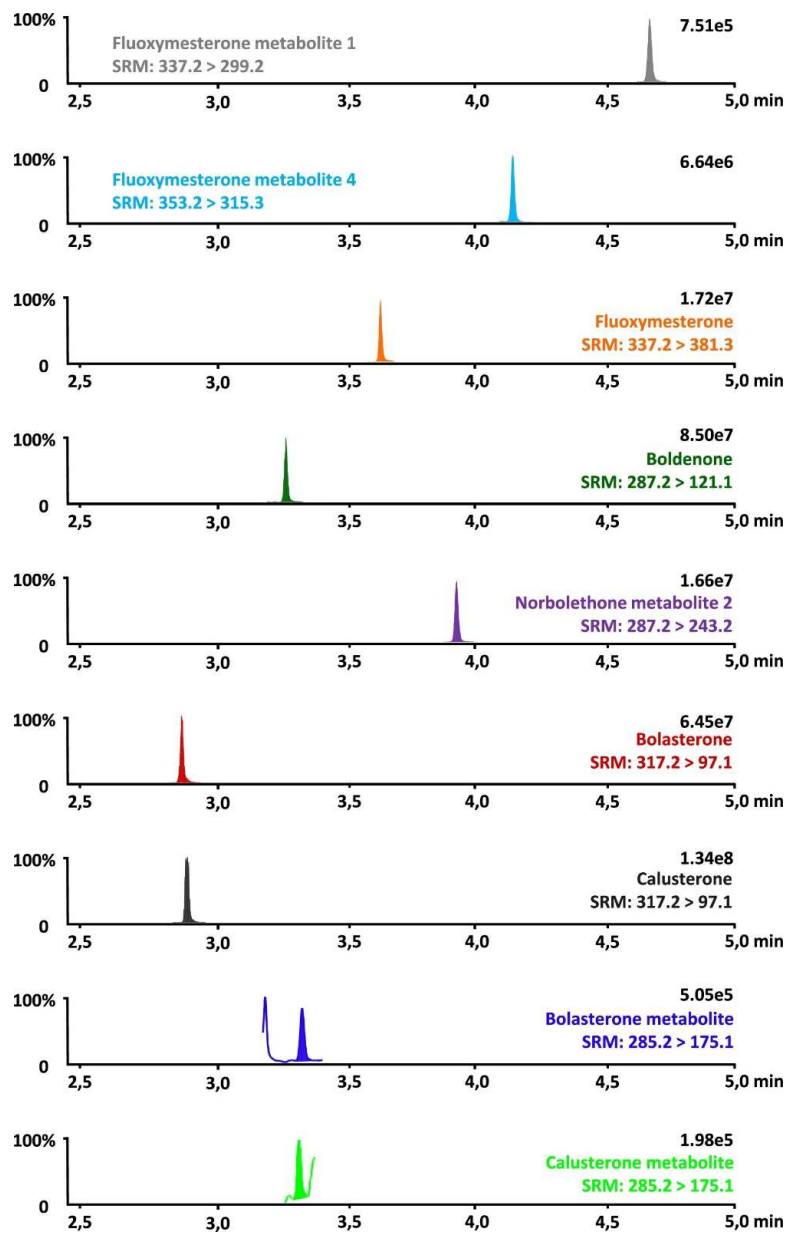


Figure 5

