

SFC-MS versus LC-MS - advantages and challenges

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For decades, chromatographers have been interested in the technique of supercritical fluid chromatography (SFC), primarily due to the rapid separations and complementary chromatographic selectivity, as well as its high 'green credentials' compared to LC. Progress in embedding SFC into routine use has been slowed by the lack of reliable SFC instrumentation. However, instrument manufacturers have recently started to invest in the development of equipment that is advanced and reliable enough to meet the demanding expectations of routine analytical laboratories.

SFC instrumentation in terms of ease-of-use and performing method development is similar to liquid chromatography (LC), reducing the barrier for user acceptance. SFC offers several potential advantages over LC regarding complementary selectivity and rapid analysis time as well as reduced solvent consumption with an environmental benefit of using CO₂. For SFC to be accepted as a valuable alternative to (U)HPLC, hyphenation to mass spectrometry (MS) is essential. Preliminary investigations have reported major gains in sensitivity when using a zero-split interface design, and in some cases a significant decrease of matrix effects with SFC-MS compared to LC-MS [1, 2].

This paper provides an overview of the advantages to be expected when hyphenating SFC with MS, and also considers the challenges that may be encountered in SFC-MS method development.

Overcoming traditional notions

One of the biggest challenges in establishing SFC as a routine analytical technique is overcoming the initial reservations people may have when looking at something they consider to be a niche technique for expert users. However, advances in technology over recent years and an increase in research in this area [3-5] are helping to remove these preconceptions. For example, Dispas et al published a paper in 2018 on a 'First inter-laboratory study of a Supercritical Fluid Chromatography method for the determination of pharmaceutical impurities' [5] that demonstrates the applicability and transferability of an SFC method with good reproducibility, as required in quality control laboratories. It shows that with development of new generation equipment reliability of the instrumentation is no longer an issue.

Similarities and differences

Scientists who are experienced in the use of (U)HPLC equipment will find that in terms of usability and application, SFC is very similar. When the two techniques are compared, many similarities are apparent: both can be used in different modes based on stationary and mobile phase characteristics, organic modifiers and additives are used to adjust selectivity, silica based porous or fused core particles can be employed and separations are run in either gradient or isocratic mode.

Some differences should also be considered, such as the compressibility of the mobile phase. In SFC, unlike LC, small changes in pressure affect fluid density and can have a strong effect on analyte retention. This is why a reliable backpressure regulator to keep the system pressure stable is a crucial part of an SFC system.

Advantages of SFC

There are also definite advantages to consider when looking at SFC for routine use, such as the lower viscosity of the mobile phase, resulting in the possibility to run higher flow rates and therefore deliver higher throughput. Cost per sample can be significantly reduced, in addition to the green credentials of the technique and its advantages in separation of isomers [6].

It also offers complimentary selectivity compared to the standard reversed phase (RP) LC approach. Peaks show very different elution patterns, even when choosing the same column, as can be seen in Figure 1 [7], showing MRM chromatograms of the analysis of 442 pesticides using a C18 column in SFC-MS (a) and LC-MS (b) respectively [7].

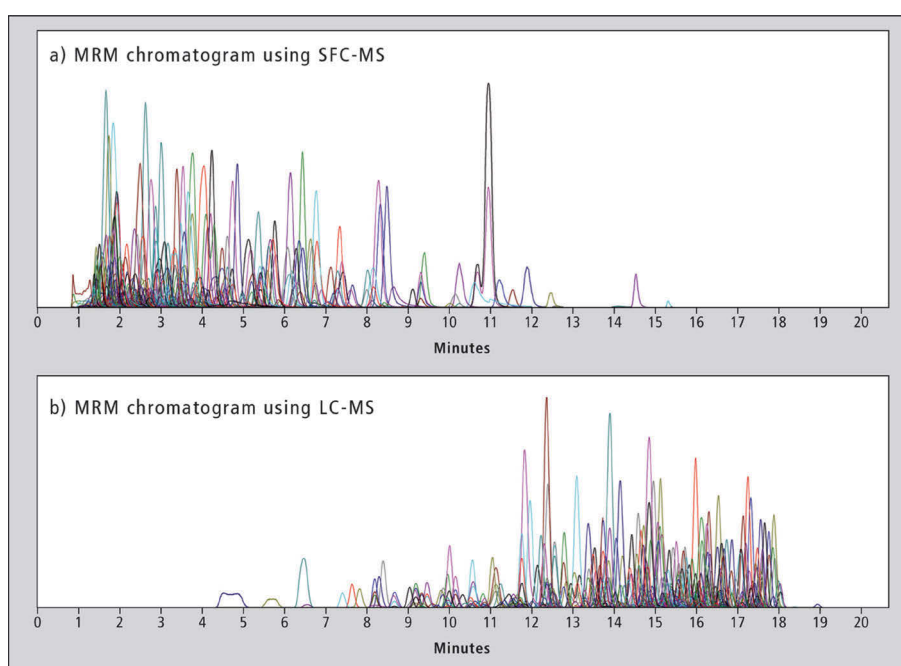


Figure 1: Comparison of elution pattern of 442 pesticides in SFC-MS vs. LC-MS (reproduced from [7]).

A closer look at the chromatograms of some highly polar compounds (Figure 2) that are difficult to retain and elute as split peak within the column dead time in RP-LCMS, reveals good retention and improved peak shape in SFC. This is just one example of the benefit complimentary SFC selectivity has to offer.

Advantageous as well as challenging

What proved to be an advantage in this case can also present a challenge. The graph in Figure 3 was generated and published by the EURL for pesticide analysis in Almeria [8]. The group looked at retention behaviour of about 160 pesticides in RP-LC as well as SFC, and they found that while retention increases on a C18 phase with increased hydrophobicity of the analyte in LC, SFC doesn't seem to exhibit an obvious elution pattern. While this offers new opportunities in terms of exploring differing selectivities for separation, it also poses a challenge in method development where there isn't a coherent rule to consider for retention behaviour. Method screening with a set of columns offering a variety of selectivities is therefore the practice of choice when developing a new SFC separation method [9].

Hyphenation to MS from SFC can also be challenging, as analytes may precipitate when the CO₂ portion of the mobile phase evaporates after the pressure is released, or the transfer line could freeze when the gas is left to expand. These issues need to be taken into account when considering a suitable interface. Direct transfer with a heated backpressure regulator (BPR) was found to be most beneficial in terms of stable spray formation, sensitivity and robustness. However, this is only possible when the BPR has a low dispersion volume to avoid causing peak band spreading in the flow line.

Most papers report a split flow design with partial mobile phase introduction through a restrictor [10], while it was found recently that by running the entire flow into the MS through a BPR with negligible volume, a significant increase in MS sensitivity can be obtained [1, 7, 8] as presented in Figure 4. This setup can also offer increased robustness of retention time and peak area, when the pressure is accurately controlled by the BPR, instead of a restrictor where pressure could be affected by changes in the mobile phase as they occur in a gradient run. When comparing the % RSD of the two approaches for retention time and peak

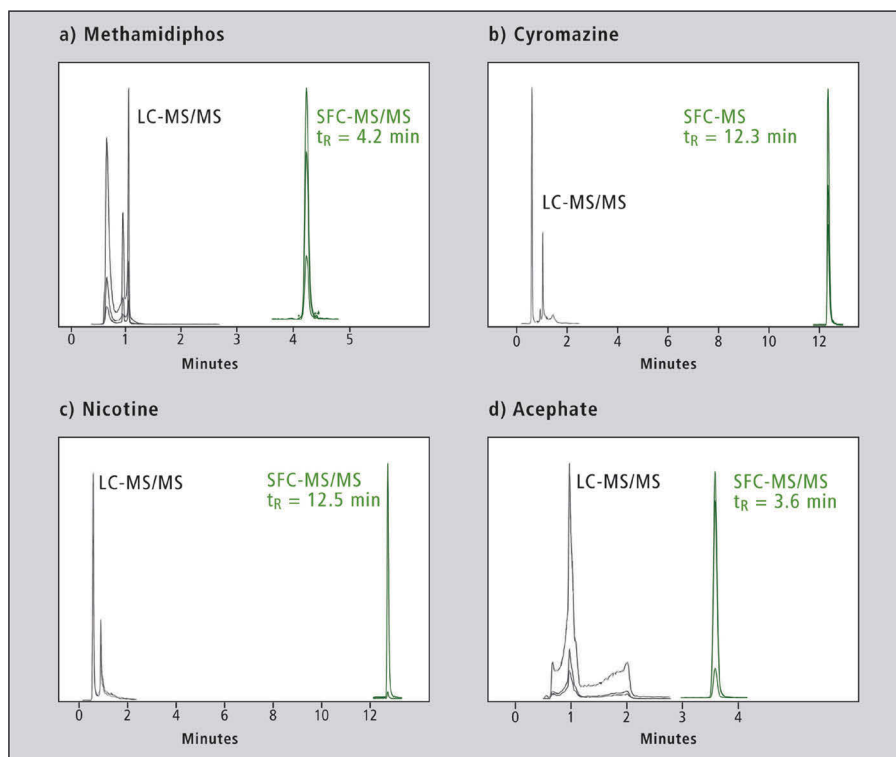


Figure 2: Comparison of SFC and LC retention for polar compounds.

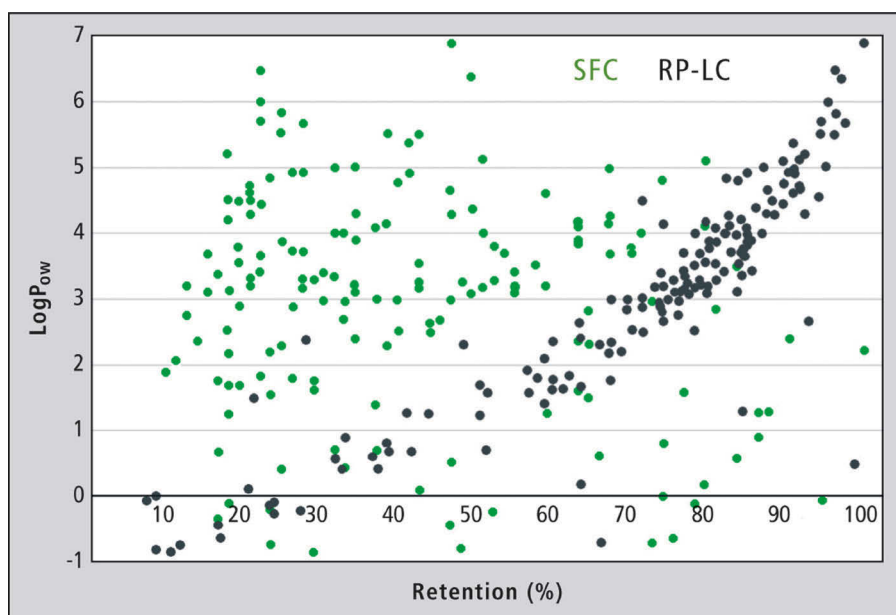


Figure 3: Retention behaviour of 160 pesticides in RP-LC compared to SFC (reproduced from [9]).

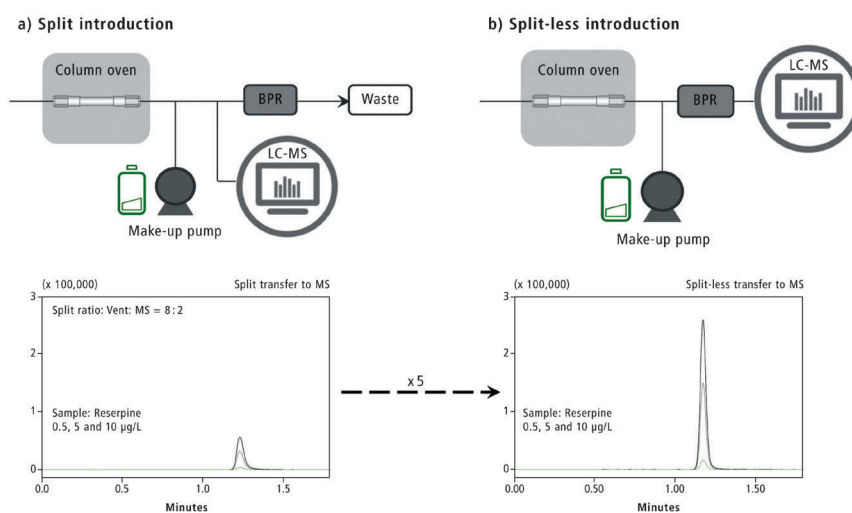


Figure 4: Comparison of sensitivity using a) restrictor and partial flow introduction into the MS and b) splitless transfer through a low-volume BPR.

	Inj. vol. (μ L)	Retention time		Area	
		Mean	% RSD	Mean	% RSD
Split	0.1	0.359	0.64	6,583	18.83
	1	0.356	0.25	81,467	4.26
	2	0.355	0.32	156,831	2.18
Split-less	0.1	0.356	0.09	16,264	6.18
	1	0.353	0.05	155,170	2.43
	2	0.350	0.07	325,739	1.16

Table 1: Comparison of % RSD for retention and peak area of Reserpine using split and no-split design interface for SFC-MS hyphenation.

area in the given example, values were found to be significantly lower in the no-split approach (Table 1).

Sensitivities

Observing that in SFC mostly gaseous CO₂ and varying portions of organic modifier are introduced into the ESI source, higher ionisation efficiency and therefore higher sensitivity can be expected due to highly efficient evaporation compared to LC with a high water content in the mobile phase. Another advantage of SFC is the possibility of using a make-up solvent to increase ionisation efficiency and possibly also spray stability, if not enough solvent is introduced with the mobile phase flow. It was found that 0.1 - 0.2 mL/min of total flow introduced into the MS was suitable for obtaining a stable spray with good sensitivity and repeatability [7].

Optimisation

With the make-up flow being introduced behind the column, it can be used to optimise ionisation efficiency without affecting chromatography. Also, pH stability of the column doesn't have to be considered in the choice of the optimum make-up solvent [1]. In a careful evaluation of MS interface parameters, it was found that unlike LC-MS where low capillary voltage gave higher peak intensity, high capillary voltage was preferable in SFC-MS. This was attributed to the high water content in LC, which is not present in SFC mobile phases [7]. It showed that MS parameters also need to be carefully optimised in order to get the most out of the technique.

After optimisation, 395 out of 442 pesticides showed better sensitivity in SFC-MS compared to LC-MS [7], which agrees with other published data [8, 11]. Matrix effects were also investigated, and in the matrices

studied (food samples) signal suppression due to co-elution of interfering matrix components was reduced significantly compared to results obtained by LC-MS, most likely due to the differences in retention. In a more in-depth study, Desfontaine et al. reported the advantage of SFC-MS over LC-MS with regards to matrix interference for the analysis of basic compounds in biological matrices. They also attributed the reduced occurrence of matrix effects in SFC-MS to an advantage in the alternate elution profile, therefore depending on the choice of separation column. In addition, it was suggested that differences in the properties of the mobile phase could lead to differences in ionisation efficiency and matrix effects [2].

Conclusion

In recent studies, SFC-MS could be established as a reliable, robust and beneficial alternative to routine LC-MS methodology, and new generation SFC-MS instrumentation prove to be very similar to LC-MS systems with regard to ease-of-use and application development.

However, retention mechanisms are not as well defined in SFC-MS as they are in LC-MS, making the method development process more empirical. Method development can still be performed quickly and efficiently using the method scouting approach. After proper optimisation, SFC-MS can offer considerable advantages in terms of separation selectivity and MS sensitivity.

SFC-MS and LC-MS should be considered as complementary techniques due to the differences in separation patterns as well as detection sensitivity, since not all compounds show higher signals when analysed by SFC.

References

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