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Review

Supercritical fluid chromatography in pharmaceutical analysis

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ABSTRACT

In the last few years, there has been a resurgence of supercritical fluid chromatography (SFC), which has been stimulated by the introduction of a new generation of instruments and columns from the main providers of chromatographic instrumentation, that are strongly committed to advancing the technology. The known limitations of SFC, such as weak UV sensitivity, limited reliability and poor quantitative performance have been mostly tackled with these advanced instruments.

In addition, due to the obvious benefits of SFC in terms of kinetic performance and its complementarity to LC, advanced packed-column SFC represents today an additional strategy in the toolbox of the analytical scientist, which may be particularly interesting in pharmaceutical analysis.

In the present review, the instrumentation and experimental conditions (i.e. stationary phase chemistry and dimensions, mobile phase nature, pressure and temperature) to perform "advanced SFC" are discussed. The applicability of SFC in pharmaceutical analysis, including the determination of drugs in formulations and biofluids is critically discussed.

Contents

1. Introduction

The availability of highly efficient analytical separation tools is essential for the pharmaceutical industry since analysis of drugs is needed at every stage of the drug development process. Various chromatographic techniques are routinely used for this purpose and among them, Supercritical Fluid Chromatography (SFC) is arousing a growing interest since few years. Although it is not perfectly correct term, because the mobile phase in SFC is not always in its supercritical state (see Section 2.2.2 for details), the same abbreviation will be used in this manuscript regardless the mobile phase state.

The use of fluids in their supercritical state was first reported in 1962 by Klesper et al. [1]. When pressurizing and heating some fluids beyond their critical point, they exhibit particular behavior as a chromatographic eluent. First, the viscosity and diffusivity of

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such a fluid is very close to those of a gas resulting in higher separation efficiency at high mobile phase velocity while maintaining a very low pressure. Secondly, their density and solvating power, which are similar to the one of a liquid, provide a good solubility and fast transportation of the analytes. Despite these interesting properties, the pharmaceutical industry showed very limited interest in SFC in these early times, and continued to use the classical and established liquid and gas chromatography (LC and GC) techniques. The SFC technique caught a second wind in the early 1980s when Novotny et al. followed by Lee et al. [2–5] introduced the concept of capillary SFC (cSFC). cSFC consisted in using capillary or open tubular columns with mobile phase being pure supercritical fluid or eventually supercritical fluid with the addition of very low proportion of co-solvent. This technique attracted particularly the GC-community which could see in it an extension of the GC possibilities. Several fluids could be considered for doing SFC, as they have P_c and T_c values that can be conveniently reached. Nevertheless, carbon dioxide (CO_2) was rapidly adopted as a the preferred supercritical fluid over others like some hydrocarbons [6,7], N_2O or ammonia [8] which presented several drawbacks with respect to safety considerations, hardware corrosion, unsuitability for thermolabile compounds and environmental impact [9]. The dipole moment of CO_2 being null, supercritical CO_2 exhibits highly lipophilic properties (similar to hexane or heptane) and its use was originally almost restricted to the analysis of lipophilic compounds. This feature was the main reason why cSFC was never considered as a viable option in the pharmaceutical field, where drug analytes and their impurities are usually highly polar and thus show a limited solubility in pure CO_2.

The first commercial packed-column SFC system was introduced in 1983 by Hewlett Packard. Contrarily to the cSFC systems which were essentially derived from GC systems, this new apparatus was actually an upgrade of an HPLC system and used packed columns similar to the ones applied in LC. This was the beginning of packed-column SFC (pSFC) [10]. Basically, pSFC differs from cSFC by the fact that packed columns are used and more organic modifier is added to the supercritical fluid to increase its polarity and broaden the range of analyzable compounds [11,12]. However, despite the obvious advantages of pSFC over LC such as higher throughput, better kinetic performance, lower solvent toxicity and environmental impact, its application in the pharmaceutical field has been limited to chiral analytical and preparative chromatography in the 1990s [13]. The reason of this narrow interest was mainly due to the poor quantitative performance as well as lack of reproducibility and robustness of the analytical systems. Limited sensitivity has also been an obstacle for the development of pSFC compared to LC. This was particularly critical for drug impurity profiling where detection of very low levels of impurities (0.1% of API) is required. Higher noise of UV baseline in SFC was mostly attributed to the refractive index variations with pressure and temperature and mobile phase compressibility [14].

Nowadays, LC still remains the gold standard for pharmaceutical analysis but there is a clear need for orthogonal techniques which could be added to the toolbox of the pharmaceutical analyst and which would assist and complement LC. GC and capillary zone electrophoresis (CZE) are too limited in terms of possible applications in the pharmaceutical field. Indeed, GC is mainly used for the analysis of residual solvents in raw material [15,16], while CE is scarcely used, except for the determination of physico-chemical properties (pK_a, $\log P$, and $\log D$) and analysis of counterions [17]. The very recent and remarkable comeback of SFC within the separation science community opens new perspectives in terms of expanding and improving the analytical toolbox of pharmaceutical analysts.

The goal of this review is to discuss the recent advances in achiral analytical SFC for pharmaceutical analysis. Moreover, considering that chiral separations and preparative SFC purification were the driving force for the revival of the SFC technique, some recent pharmaceutical applications in these two domains will be also presented in this review.

2. Instrumentation and experimental conditions for advanced analytical SFC technology

2.1. Advanced SFC instruments

The renewed interest in SFC seen in the pharmaceutical analysis community during the last couple of years is mainly driven by the introduction of new state-of-the-art systems by a number of key instrument manufacturers. The design of the newly developed devices is essentially based on recent UHPLC instruments, which already incorporated the elements which overcome several challenges that prevented SFC from being a robust technique. First, these new SFC devices are compatible with the last generation of columns to get the highest kinetic performance (efficiency and throughput). These columns are made of fully porous silica particles with particle size up to below 2 μm or superficially porous silica particles (also called core–shell particles) of less than 3 μm. Both types of columns may generate higher pressures than the traditional columns packed with 5 μm particles, especially when working with high amount of organic modifier. Secondly, the dead volumes of the new SFC systems have been lowered to limit band broadening due to the system itself and fit with the highly efficient columns mentioned above. Finally, the systems have improved in terms of reliability, quantitative performance and sensitivity compared to the past generation of SFC devices. For this purpose, a special care was brought on the design of the backpressure regulator (BPR) which controls the pressure in the system, and on the CO_2 delivery system. This limits mobile phase density variations. The following paragraphs describe some of the features of two new advanced SFC systems which have been recently commercialized. Besides the two, other providers (Pic, Jasco, Shimadzu, etc.) are offering conventional SFC instrumentation.

The ACQUITY Ultra Performance Convergence Chromatography (UPC^2) system was introduced in 2012 by Waters (Milford, MA, USA). Its design is clearly inspired from the Acquity UPLC system. The extra-column volume and dwell volume were estimated at 59 μL and 440 μL, respectively [18]. It is equipped with a dual-stage BPR including a passive component maintaining a pressure of 104 bar and the active component to further increase the backpressure, enabling a better pressure control within the system. The compression and chilling of CO_2 take place inside the pump thanks to an integrated device which is preferable to older SFC systems where this unit was separated and stands outside the reconfigured HPLC pump (the path between chilling unit and the pump can lead to density variations) [19]. The pump heads cooling is independent and achieved by a Peltier module. In consequence, good control of both pressure and temperature, and thus density, are achieved resulting in improved reliability, repeatability and sensitivity. The maximum flow rate and pressure of the instrument are 4 mL/min and 413 bar, respectively. However, the maximum pressure decreases linearly to 293 bar in the flow rate range from 3.25 to 4 mL/min. The stainless steel UV flow cell of 8 μL and 10 mm path length is also adapted to pressure up to 400 bar. The improvement in sensitivity is also enhanced by the adaptation of the photodiode array (PDA) detector to supercritical fluids. The difference in refractive indices (RI) between CO_2 and methanol (the most-commonly used co-solvent), 1.00 and 1.33 respectively [19], is compensated by the use of high-strength silica lenses instead of sapphire ones usually encountered in reversed phase HPLC–UV-detectors where there is nearly no difference between RI of the components of the mobile phase. All these features lead to significant improvement