Title: **Alternative chiral and achiral solvents in supercritical fluid chromatography**

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Supercritical fluid chromatography (SFC) has been an emerging technique since a few decades ago as laboratories started to consider more environmentally friendly separation techniques. SFC is already considered a fairly green technique since the major component of the mobile phase is supercritical CO_2 ($scCO_2$), which is not toxic, compared to normal phase liquid chromatography (NPLC) that uses toxic solvents, such as hexane. To make SFC "greener", the co-solvents used to increase the polarity of the mobile phase, such as methanol or acetonitrile, should be replaced by other solvents considered "green", for instance, ionic liquids, deep eutectic solvents, and bio-derived solvents. Therefore, this report aims to (1) research into existing green solvents, by means of literature study, which have already been applied or have chances to be applied in SFC as well as other separation techniques; and to (2) assess the potential of a bio-based solvent, Cyrene, in achiral and chiral separations.

A supercritical fluid chromatography system consists of different modules that work together to ensure the performance of the analyses. All modules have to endure the high pressures that have to be applied to ensure that the gas remains in supercritical conditions. A clear difference in instrumentation compared to a high-pressure liquid chromatography (HPLC) is the use of a backpressure regulator, which controls the outlet pressure of the system in order to prevent the fluid from expanding through.

Through the history of SFC, a lot of fluids have been used as the mobile phase, such as N_2O or NH_3, but its application has been discontinued due to issues regarding flammability, high critical temperatures, and safety issues. As a result, the most common used fluid is $scCO_2$ as its use yields a number of advantages which include low price, UV transparency, availability, non-toxicity, and the fact that it is considered a green solvent. Furthermore, $scCO_2$ allows for separations at relatively mild conditions in SFC as there is no need for excessively high pressures and temperatures, as its critical point is located at 31.3°C and 72.8 atm.

Supercritical CO_2 presents an eluotropic strength similar to hexane, thus needs the addition of polar solvents to elute high polar compounds. The conventional modifiers are methanol, ethanol, isopropanol, and acetonitrile. Since they are not respectful enough with the environment, green alternatives have been approached. Some examples of environmentally friendly solvents that have been used in SFC and other separation techniques are bio-based solvents, ionic liquids, deep eutectic solvents, and supercritical fluids. The green solvent Cyrene has not been used in SFC, thus this is the goal of this report.

Before starting the assessment using Cyrene, an optimisation method was developed using methanol as well as a buffered methanol with 0.1% of formic acid as the modifier. For the achiral analysis, a sample with toluene, aniline, pyridine, propylparaben and caffeine was prepared; and for the chiral analysis, the sample contained three racemic compounds: trans-stilbene oxide, benzoin and ketoprofen. The analyses were performed with isocratic and gradient conditions, and it was observed that the peaks were better resolved when using a concentration of 5% of buffered methanol with isocratic conditions in both achiral and chiral separations.

Since Cyrene is a chiral molecule, it might offer the possibility to be able to be used as a chiral solvent. However, using pure Cyrene presented issues in terms of viscosity and absorbance due to its high density (1.25 g/ml) and not being completely colourless (light yellow). Therefore, two different mixtures of MeOH:Cyrene were prepared in order to reduce the viscosity.

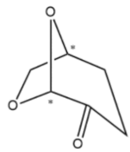

Figure 1. Structure of Cyrene.

No peak splitting was observed when using a mixture with a concentration of MeOH:Cyrene (90:10), while it was observed when using a mixture containing a higher concentration of Cyrene (75:25). Thus, by increasing the amount of Cyrene, chiral separations might be plausible, although some technical issues might be occurring at the same time since three peaks appear for one only compound.

Further research must be done in order to affirm the possibility of Cyrene to be used as a chiral solvent, as well as the possible enhancement in combination with a chiral column.

Moreover, future research regarding the use of Cyrene in other separation techniques, e.g. RP-HPLC, might yield interesting results as it has been shown that Cyrene is miscible in water as well as in methanol or acetonitrile, which would make it highly applicable in HPLC.