Technical Note

Excellent peak symmetry in chiral SFC: methanesulfonic acid as promising additive

Supercritical fluid chromatography (SFC) employs supercritical carbon dioxide combined with an organic modifier as the mobile phase. To optimise performance, additives such as ammonia (NH_3), diethylamine (DEA), formic acid (FA), or trifluoroacetic acid (TFA) are often used to improve peak shape and resolution.

Peak symmetry is a critical factor in chiral separations, particularly when multiple peaks elute closely together. Strong polar interactions or flexible molecular structures often lead to peak tailing, reducing resolution and affecting quantification. The strategic use of mobile phase additives offers an effective solution to these challenges, ensuring high precision and reproducibility.

This Technical Note, based on the study by Spelling et al. [1] at AstraZeneca, highlights the advantages of methanesulfonic acid (MSA) as an additive in chiral SFC. The results demonstrate that MSA significantly improves peak shape compared to commonly used additives, enhancing both resolution and retention time consistency.

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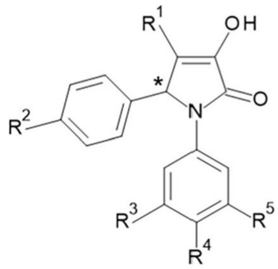
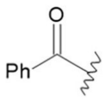
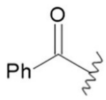
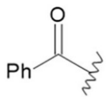
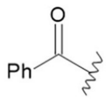
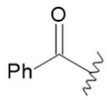
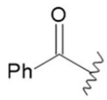
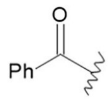
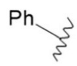
Table 1: Chromatographic conditions [1].

Columns:	CHIRAL ART Cellulose-SZ (3 µm) 150 x 4.6 mm ID CHIRAL ART Amylose-SA (3 µm) 150 x 4.6 mm ID
Part Nos.:	KSZ99S03-1546WT KSA99S03-1546WT
Eluent:	A) CO_2 B) methanol + 20 mM additive
Additives:	Methanesulfonic acid (MSA) Diethylamine (DEA) Trifluoroacetic acid (TFA) Formic acid (FA)
Gradient:	40 %B isocratic (CHIRAL ART Cellulose-SZ) 5–40 %B (0–5 min), 40 %B (5–10 min) (CHIRAL ART Amylose-SA)
Flow rate:	3.5 mL/min
Temperature:	40 °C
Detection:	UV at 210 nm
Backpressure:	120 bar
Sample:	8 model compounds (AstraZeneca in-house) Dissolved in methanol at about 1 mg/mL



	1	2	3	4	5	6	7	8
R^1								
R^2	$-NO_2$	–H	$-NH_2$	–H	–	–H	–	$-NO_2$
R^3	–COOH	–H	–COOH	–COOH	–	–H	–	–H
R^4	–OH	–H	–OH	–OH	–	–H	–	–OH
R^5	–H	–H	–H	–H	–	$-OCH_3$	–	–COOH

Compounds 5 and 7 are not in the public domain

Figure 1: Model compounds used in the study [1].

Among the tested columns, the **CHIRAL ART Cellulose-SZ** column provided the highest number of well-resolved peaks. With conventional additives such as DEA, TFA, and FA, achieving optimal peak symmetry—particularly for challenging compounds like compound 4 (Figure 2)—remains difficult. In contrast, MSA delivers superior peak shapes and reduces retention times.

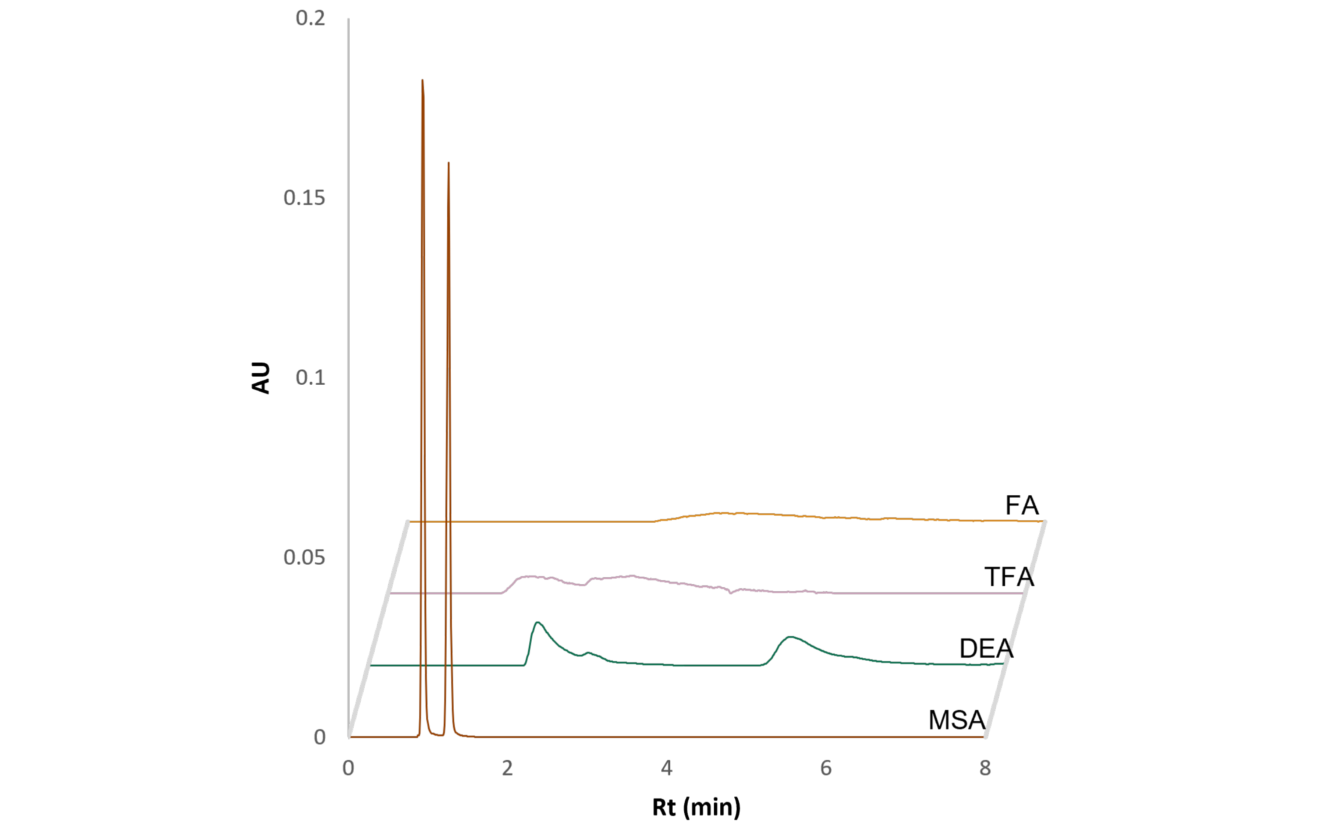


Figure 2: Comparison of the different additives used for separating compound 4 with CHIRAL ART Cellulose-SZ [1].

The **CHIRAL ART Amylose-SA** column demonstrated outstanding results with MSA, surpassing other additives in delivering improved peak symmetry and resolution across all 8 compounds. Figure 3 illustrates a direct comparison between DEA (A) and MSA (B). With MSA, peaks are significantly sharper, achieving complete separation for 5 compounds, whereas DEA only partially resolves 2 compounds.

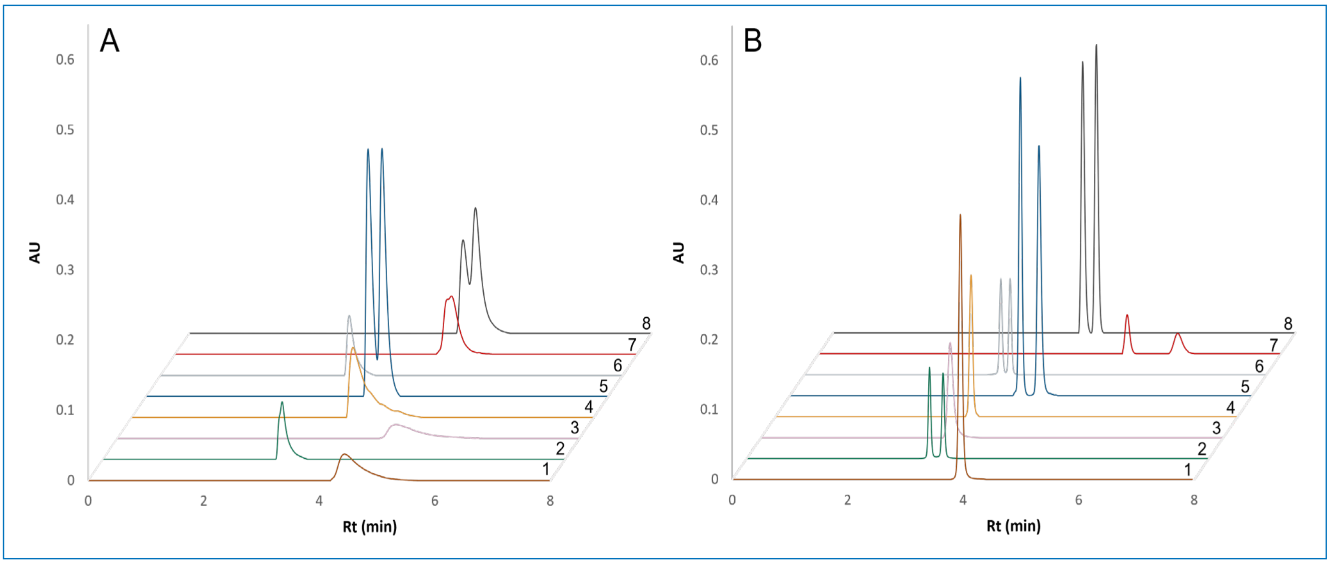


Figure 3: Comparison of DEA (A) and MSA (B) as additive using the CHIRAL ART Amylose-SA column for all eight compounds [1].

Conclusion

Combining MSA with high-performance CHIRAL ART columns has the potential to further enhance resolution in SFC. Additionally, MSA offers practical advantages over commonly used TFA, being less hazardous and easier to manage due to its lower volatility. However, comprehensive data on the long-term stability of systems and column hardware when exposed to this strong acid remains limited, highlighting the need for further investigation.

References
[1] Spelling, V., Buica, A. & Leek, H. Methanesulfonic acid in chiral supercritical fluid chromatography: the quest for perfect peaks and the trials of resolution. Discov. Chem. 1, 68 (2024). https://doi.org/10.1007/s44371-024-00058-y