Increasing Focus on Greener Eluents in Preparative Chromatography

Background

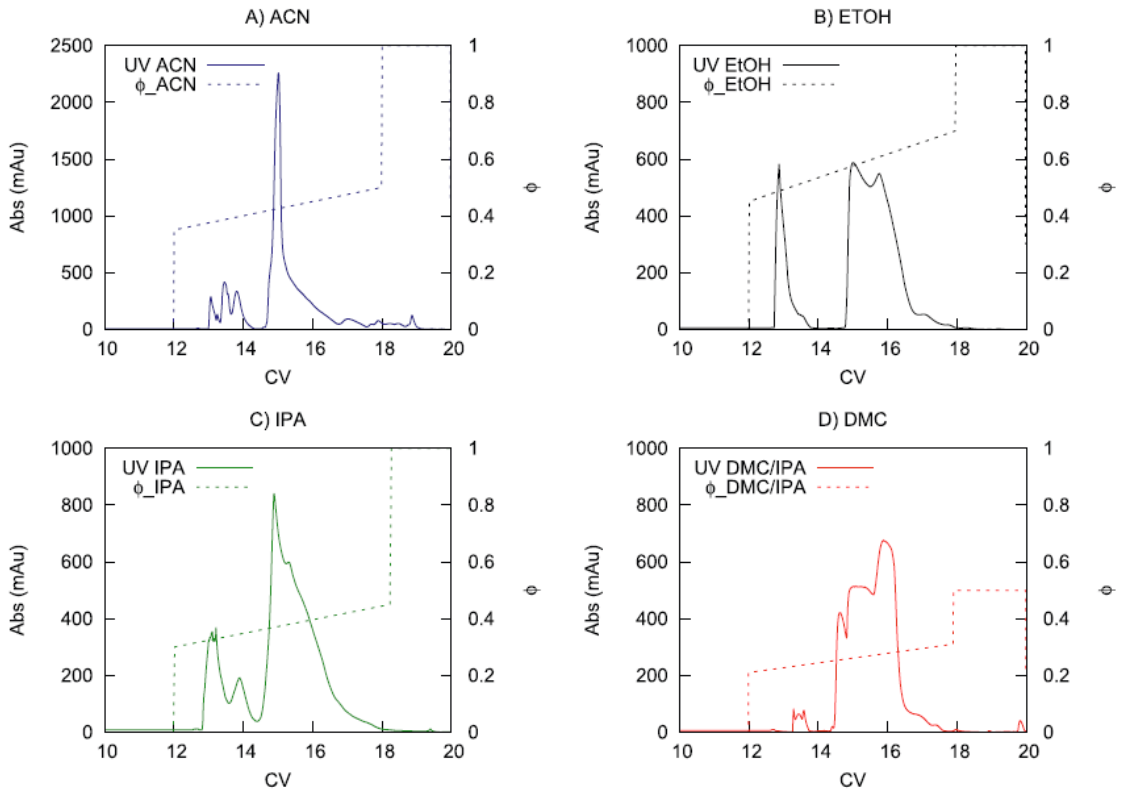
Preparative liquid chromatography is a key technique for the isolation and purification of bioactive compounds from complex matrices. At the same time, it is traditionally associated with high consumption of organic solvents, leading to both environmental and economic challenges. In recent years, a clear trend has emerged toward the use of more sustainable, so-called “green” eluents. The goal is to reduce environmental impact, improve operator safety, and maintain chromatographic performance. Modern stationary phases enable robust and reproducible separations even under varying solvent conditions. This robustness is essential when evaluating alternative, greener eluents at preparative scale. The data presented in this application note are derived from a recently published scientific study on the use of green solvents in preparative chromatography, ensuring a robust and experimentally validated basis for the discussion. The original publication is referenced below.

Challenge: Conventional Eluents and Their Limitations

Acetonitrile is one of the most commonly used organic modifiers in reversed-phase liquid chromatography. It offers high elution strength, low viscosity, and excellent miscibility with water. However, it also presents significant drawbacks, including toxicity and the cost and complexity of handling and disposal. These issues become particularly critical at preparative scale due to the large solvent volumes involved. Driven by Green Chemistry principles, alternative solvents are increasingly being explored. Ethanol, isopropanol, and dimethyl carbonate represent promising options due to their improved environmental and safety profiles. Mixed systems, such as dimethyl carbonate combined with isopropanol, are of particular interest, as they provide high elution strength while reducing overall solvent consumption.

Experimental Setup: Melittin Purification

The data presented in this Application Note are based on a preparative reversed-phase separation of a bioactive peptide (melittin) from a complex natural matrix (honeybee venom). The purification was performed on a YMC-Triart Prep C18-S column under identical operating conditions while varying only the organic modifier in the mobile phase.
As shown below four eluents were compared: acetonitrile (ACN), ethanol (EtOH), isopropanol (IPA), and a mixed system of dimethyl carbonate and isopropanol (DMC/IPA). Due to the limited water miscibility of DMC, IPA was added as a co-solvent to obtain a homogeneous mobile phase composition. Without a co-solvent, the applicability of DMC in aqueous mobile phases would be significantly limited.Key performance indicators included purity, yield, productivity, and solvent consumption, allowing a direct comparison between conventional and greener solvent systems.



Column:	YMC-Triart Prep C18-S (10 µm), 250x4.6 mm ID	Detection:	UV at 280 nm
Eluent:	A) water + 0.1% TFA	Injection:	41.5 µL
	B) different organic modifier + 0.1 % TFA	Sample:	lyophilized honeybee venom
Flow rate:	1.0 mL/min		

The greenness of each method was assessed using established green metrics such as AGREE, AMGS, ChlorTox, and CHEMS-1. These approaches combine factors including solvent toxicity, consumed volume, energy demand, and environmental and health hazards into a comparative score, enabling a quantitative evaluation of how sustainable a chromatographic process is.

Performance vs. Sustainability and the Impact on Processes

The table below summarises the key results obtained from the preparative melittin purification on a YMC-Triart Prep C18-S column:

Eluent	Purity (%)	Yield (%)	Solvent Consumption	Greenness	Assessment
ACN	~96–98	~50	high	low	reference
EtOH	~94–95	~47	very high	good	limited
IPA	~95	~87	reduced	good	very good
DMC/IPA	~96	~83	strongly reduced	very good	best option

The results clearly show that isopropanol and especially the DMC/IPA mixture provide an excellent balance between chromatographic performance and sustainability, while significantly reducing solvent consumption compared to ACN. Switching to greener eluents requires balancing chromatographic performance with sustainability. While acetonitrile often provides the best peak shape, alternative solvents can achieve comparable purities while offering clear advantages in terms of environmental impact and solvent consumption. The choice of eluent directly affects key process parameters such as purity, yield, productivity, and solvent consumption. In this application, the use of greener eluents on YMC-Triart Prep columns enabled high recovery of the target compound while reducing environmental impact.

Conclusion

This application demonstrates that greener eluents can be successfully implemented in preparative chromatography without compromising performance when using robust stationary phases. Green solvents such as ethanol, isopropanol, and especially dimethyl carbonate represent viable alternatives to conventional solvents like acetonitrile. The combination of DMC and IPA in particular enables reduced solvent consumption, high yields, and excellent purity. The results underline the shift from purely performance-driven method development toward a more holistic approach that integrates sustainability.

This Application Note is based on data published by the University of Ferrara iin the following publication:
[1] Tabesh et al., J. Chromatogr. A 2025, 1763, 466460.

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