Greener Chromatography Solvents

Environmentally Friendly Greener Alternatives

Ethyl acetate/Ethanol 3:1 (v/v) Solution

A Greener Chromatography Alternative to Dichloromethane

Sigma-Aldrich is delighted to offer Ethyl acetate/Ethanol 3:1 (v/v) solution (Cat. No. 745588), a novel green solvent alternative developed to replace dichloromethane (DCM) in flash chromatography purification, TLC and related HPLC methods. This Greener Chromatography brochure was developed to provide a quick benchtop chromatography reference guide of the relative eluting strengths of Ethyl acetate/Ethanol 3:1 (v/v) solution required to resolve either neutral compounds, basic compounds or acidic compounds (**Figure 1**). A demonstration of this greener chromatography and its other advantages, such as better baseline separation of neutral compounds, has also been included (**Figure 2 & 3**).

Cat. No.	Product Description
745588-4x4L	Ethyl acetate/Ethanol 3:1 (v/v) solution, (Ethyl acetate solution with 26.2% v/v SDA 35A), for HPLC
34873-4x4L	Heptane, for HPLC, ≥99%

Benefits of Ethyl acetate/Ethanol 3:1 (v/v) solution as a Dichloromethane Replacement

- Comparable separations of Neutral, Basic and Acidic Compounds (**Figure 1**)
- Chromatographic improvement in baseline separation of compounds (**Figure 2 & 3**)
- No interference from chemical stabilizers used by dichloromethane
- Reduced chlorinated waste containment and hazardous disposal costs
- Significant reduction in environmental impact

In medicinal chemistry, chromatography is commonly used to analyze and purify a wide range of organic molecules. However, chromatographic solvents generate the largest component of medicinal chemistry waste. Because of DCM's toxicity to human and its significant environmental impact, users are also affected economically. Users not only have to pay ever climbing cost of the high purity DCM, they are again required to pay high fees for its disposal.

In 2012, Taygerly at Amgen developed an empirically-derived green solvent selection guide[1] to aid chemists in choosing greener solvents for chromatographic purification. In particular, the use of heptane (nonpolar eluent) combined with an Ethyl acetate/Ethanol 3:1 (v/v) solution Cat. No 754488 (polar eluent) is a suitable solvent replacement for DCM/MeOH mixture.

Greener Chromatography Solvent Selection Guide Development

The following information highlights a quick review of details used to establish Ethyl acetate/Ethanol 3:1 (v/v) solution's usefulness as a green solvent alternative for dichloromethane (DCM) in flash chromatography purifications[1, 2] and related HPLC methods.

Compound selection

Various green chromatography solvent mixtures are used to evaluate the purification of "druglike" molecules that medicinal chemists regularly prepare and purify. Thin-layer chromatography (TLC) is employed to evaluate alternative green solvent systems considered to be greener alternative to dichloromethane. Selected compounds were classified into three families: Neutral, Basic and Acidic Compounds. (**Figure 4**)

- Neutral compounds selected do not contain a carboxylic acid or an aliphatic amine that would require acidic or basic additives to avoid tailing or streaking during elution.
- Basic compounds contain tertiary aliphatic amines and a basic solvent additive (NH_4OH) was necessary to prevent tailing.
- Acidic compounds contain carboxylic acids, and an acidic solvent additive (AcOH) was necessary to prevent tailing.

Greener Chromatography Solvent Selection Evaluation Method

The relative eluting strength of a particular solvent mixture was determined by TLC analysis of the test compounds. All compounds were spotted in parallel on a single TLC plate, and the compound set was eluted with a specific solvent mixture. The retention frequency (Rf) value was measured for each individual compound. Then, the individual Rf values were averaged to give an average retention frequency (Rf_{avg}) value for the compound set in that specific solvent mixture. Solvent mixtures were systematically evaluated at varying concentrations of polar eluent. (**Figure 1**)

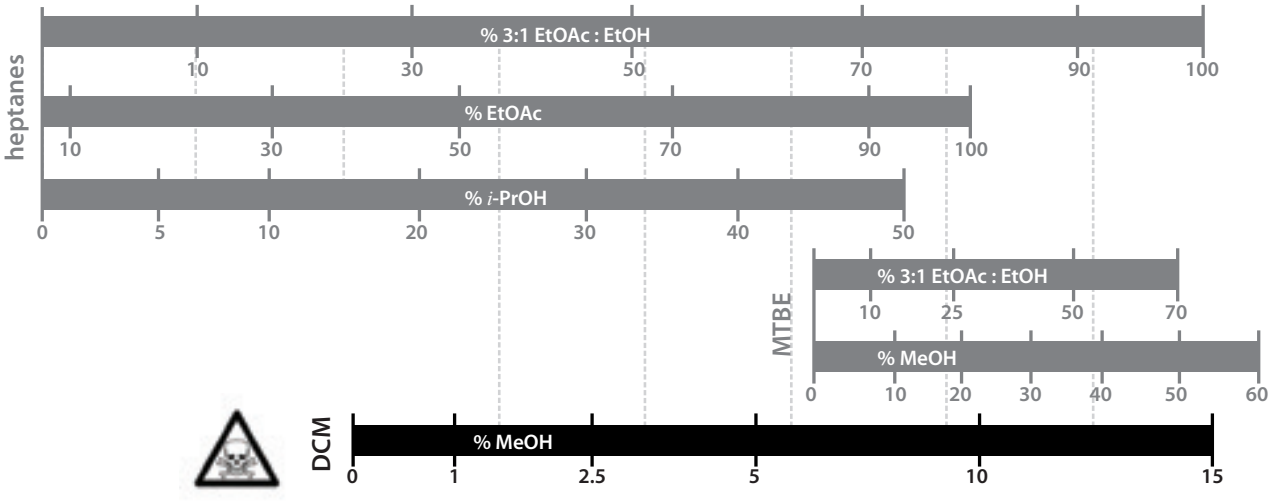
Stock green solvent blends

Laboratory efficiency was the other key requirement needed to make a successful greener alternative to DCM. The acceptance and adoption of a greener alternative by users, must be just as easy to obtain and use as its predecessors. Sigma-Aldrich worked with its developers to provide a single blend that has been manufactured to provide the end user with the same lot-to-lot consistency and performance reproducibility of an individual high purity solvent.

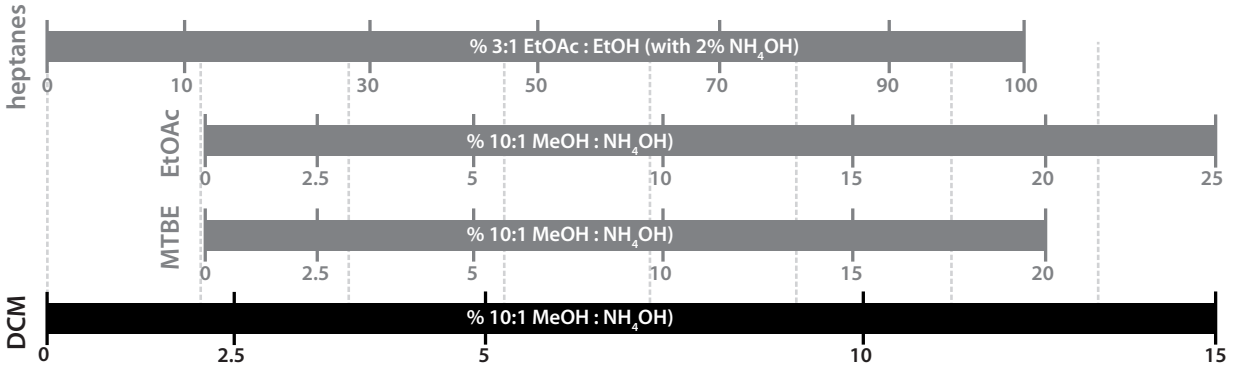
Sigma-Aldrich is proud to offer this greener solvent alternative to DCM and remains committed to enabling science to improve the quality of life.

Relative Eluting Strengths of Green Chromatography Solvent Mixtures

Neutral Compounds



Basic Compounds



Acidic Compounds

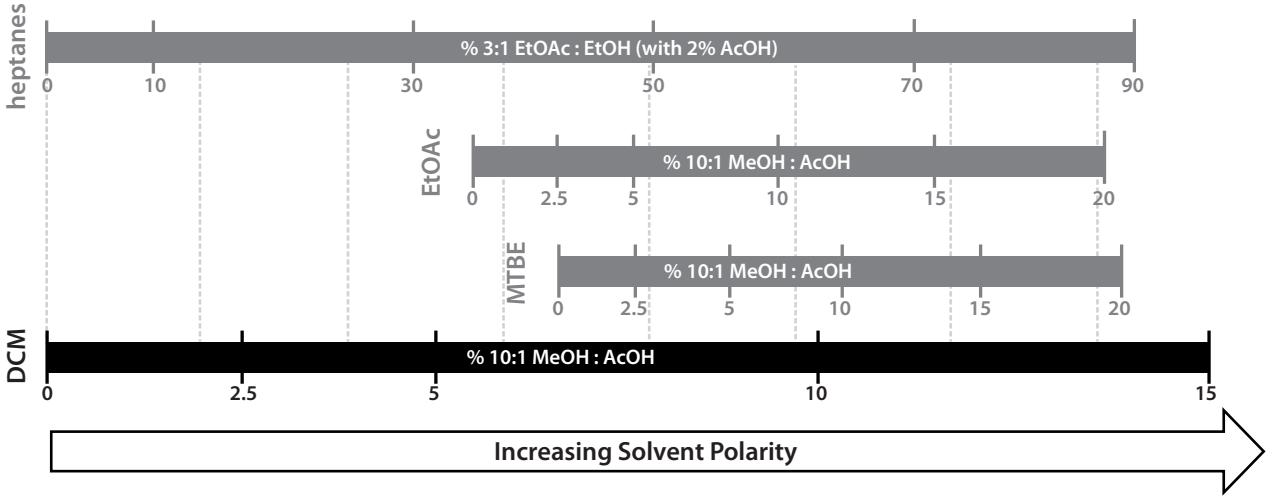


Figure 1 Green Chromatography Solvent Selection Guide. Starting from the appropriate DCM–MeOH concentration, compare vertically across the bar chart to identify greener solvent mixtures of similar eluting ability[1].

TLC and HPLC Comparison Data

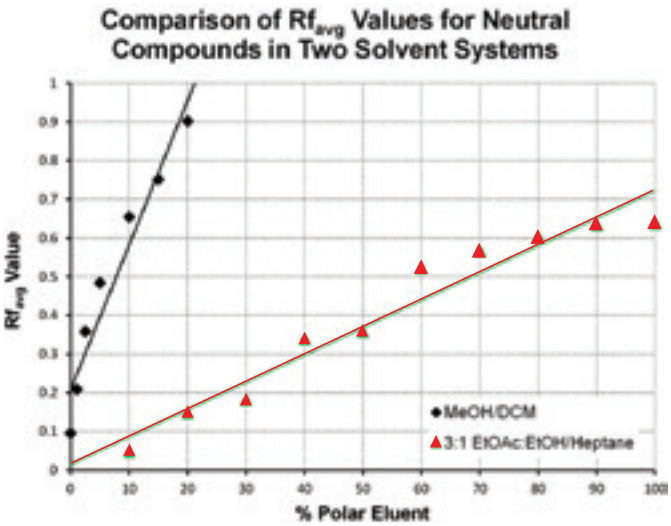


Figure 2 Shows the results of the neutral compound set analyzed in two different solvent mixtures (MeOH in DCM and 3 : 1 EtOAc : EtOH in heptanes) over a range of eluent concentrations. The individual regression lines can be compared horizontally to generate eluting strength relationships between the two solvent systems. For example, the neutral compound set was eluted with a Rfavg value of 0.5 in both 7% MeOH in DCM and in 65% 3 : 1 EtOAc : EtOH in heptanes, and thus the two solvent mixtures are considered to have similar eluting strength at these concentrations.

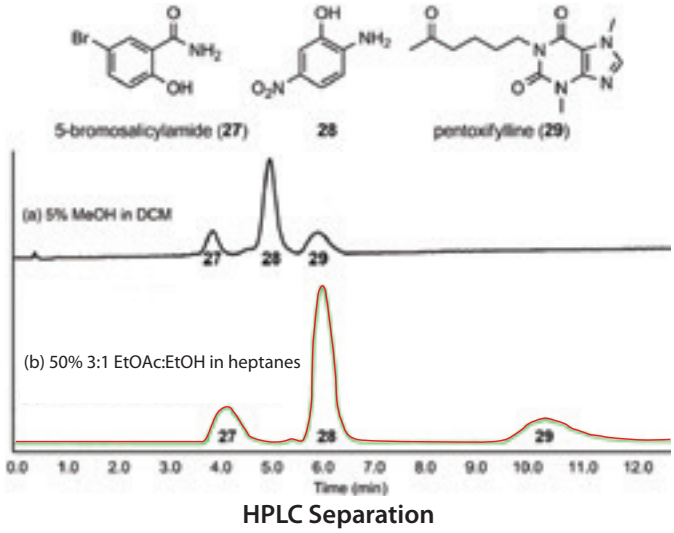


Figure 3 UV traces generated from the chromatographic separations showing the separation of a mixture of three compounds in two solvent systems: (a) isocratic 5% MeOH in DCM and (b) isocratic 50% 3 : 1 EtOAc : EtOH in heptanes. The relative eluting order of the three compounds was maintained in both solvent mixtures, and full baseline separation of compound peaks was achieved in both cases. In this particular example, peak separation was even improved in the green solvent mixture compared to the MeOH in DCM system

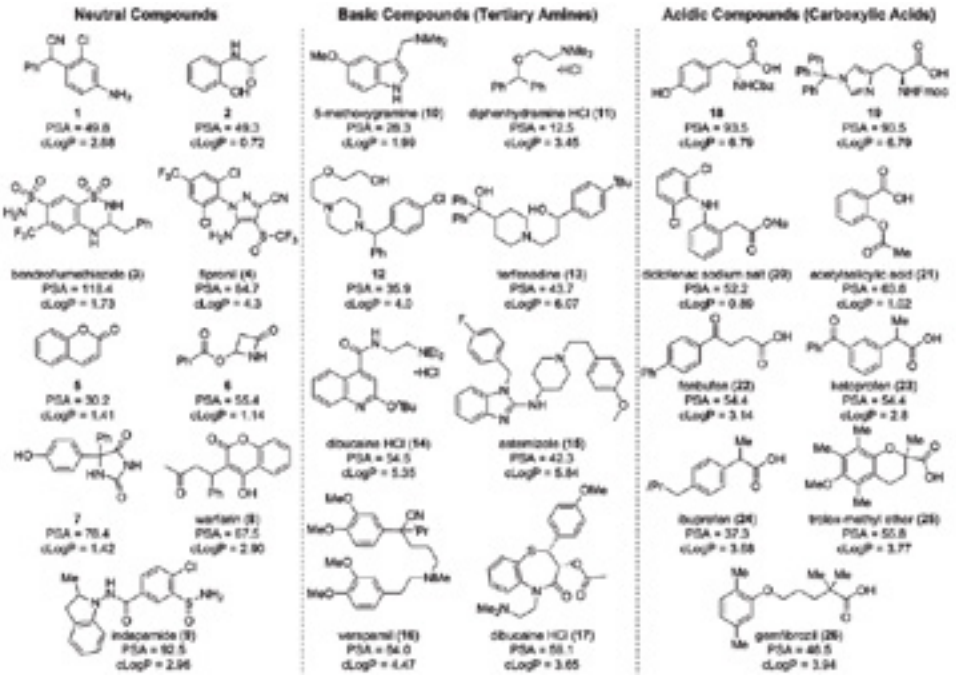


Figure 4 Shows the compounds that were selected and used to evaluated the different solvent mixture by TLC

Reference:

1. A convenient guide to help select replacement solvents for dichloromethane in chromatography; Taygerly, J. P.; et. al. *Green Chem.* **2012**, *14*, 3020
2. Sustainable Practices in Medicinal Chemistry: Current State and Future Direction; Bryan, M. C.; et. al. *J. Med. Chem.* ***2013***, *56,* 6007

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World Headquarters
3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

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