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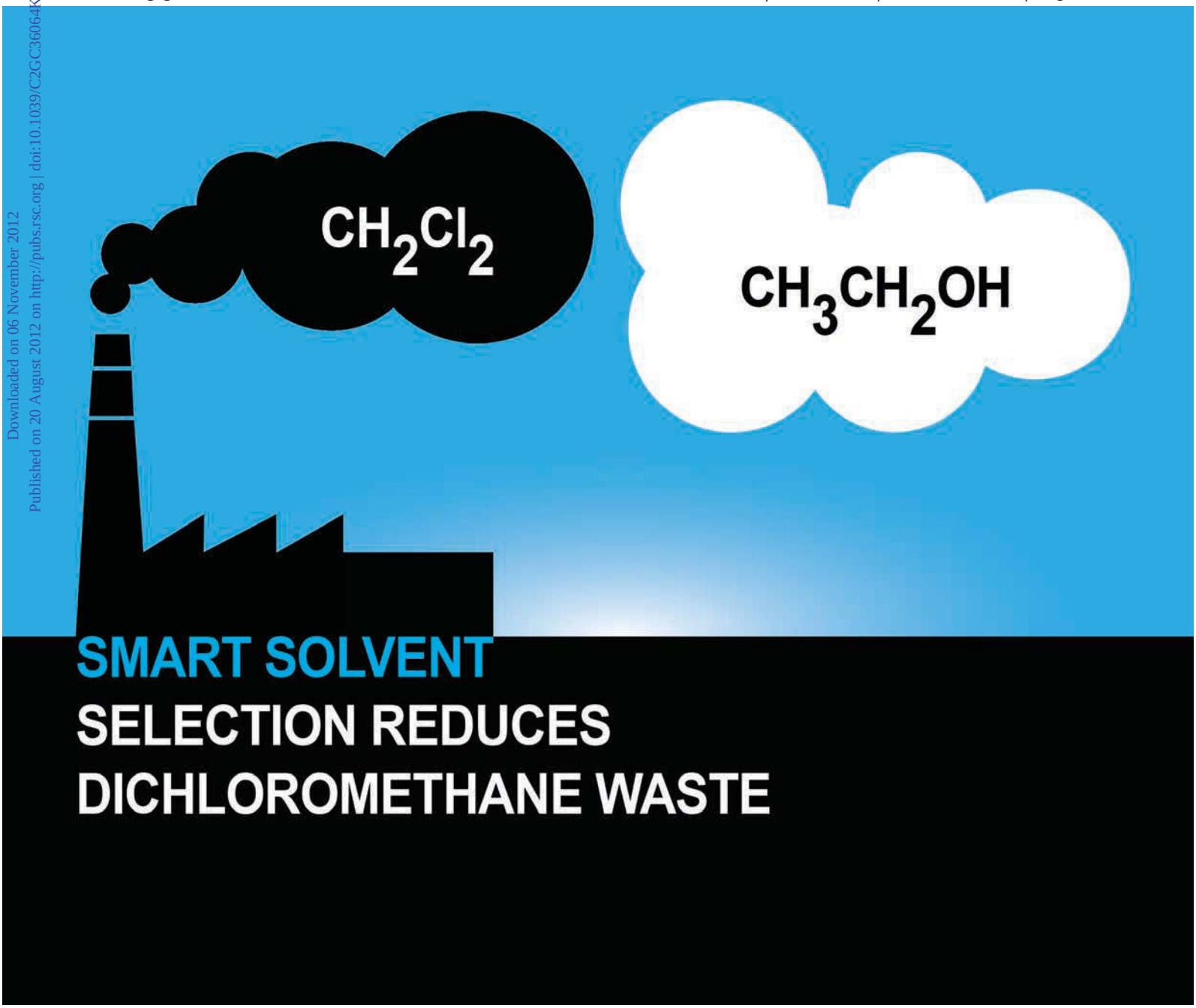

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PAPER

A convenient guide to help select replacement solvents for dichloromethane in chromatography†

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One of the largest contributors to chlorinated solvent waste in medicinal chemistry is chromatography. A set of "drug-like" compounds was employed to compare the relative eluting strengths of greener solvent systems. Disclosed herein is an experimentally-derived solvent selection guide to aid chemists in choosing greener solvents for chromatographic purification, with a particular focus on reducing dichloromethane usage.

Introduction

Green chemistry has become a highly valued practice in the pharmaceutical industry, with many manufacturing processes undergoing E-factor or Process Mass Intensity (PMI) evaluations.[1] As Green chemistry efforts have gained traction within the pharmaceutical industry, these practices have proven advantageous at earlier stages in drug discovery research, such as medicinal chemistry. While the operational scale within medicinal chemistry is smaller than that of process chemistry and manufacturing, the cumulative output of waste can be significant. Thus, the implementation of green chemistry practices in medicinal chemistry can have environmental, safety, and cost benefits. However, despite the advantages of applying green chemistry practices within medicinal chemistry, the uptake has been slower than in process and manufacturing. This is likely because of key differences in the way medicinal chemistry, process chemistry and manufacturing disciplines are executed. In particular, medicinal chemistry requires the rapid synthesis of many structurally diverse molecules, while process chemistry and manufacturing focus on the synthetic optimization of a specific target molecule to reduce overall cost and waste generation. This focused opportunity for optimization aligns well with green chemistry principles, whereas synthetic routes in medicinal chemistry are intentionally designed to be divergent, and less effort is spent on optimization. Additionally, the diversity of chemical reactions utilized in medicinal chemistry often causes difficulty in tracking metrics, obscuring the degree of green chemistry implementation.

Despite these challenges, there are unique opportunities to introduce green chemistry practices into medicinal chemistry labs. Chromatographic purification is frequently used by synthetic chemists, because this technique is broadly successful for the purification of a wide spectrum of organic molecules. Indeed, the largest component of the medicinal chemistry waste stream is spent solvent generated during chromatographic purification. Although many solvents can be utilized for effective silica gel chromatography, chemists often rely on two binary solvent mixtures for a majority of purifications: dichloromethane–methanol (DCM–MeOH) and alkanes–ethyl acetate (EtOAc). Because of this bias, the largest contributor to chlorinated solvent waste in medicinal chemistry is DCM used for chromatography. DCM is associated with both acute and chronic toxicity in humans, including respiratory toxicity, central nervous system toxicity, cardiovascular toxicity, carcinogenicity and genotoxicity. Additionally, DCM persists in the environment with a half-life of over 18 months in water.[2] The significant human and environmental toxicities associated with DCM make it important to reduce the use of this undesirable solvent. Although several tools, such as solvent selection guides[3] and reaction guides,[4] have been published to promote green chemistry practices, these have not specifically addressed the use of greener solvent alternatives in chromatography. Numerous experimental and theoretical analyses of the relative polarities of individual solvents and binary mixtures have been reported, but these have generally not been targeted to medicinal chemists and again have not focused on greener solvents.[5]

With this in mind, we have developed a green chromatography solvent selection guide intended for use as a quick benchtop reference for medicinal chemists looking for greener solvent alternatives to replace DCM–MeOH in chromatography (Fig. 4).[6] This guide should also prove useful to other synthetic

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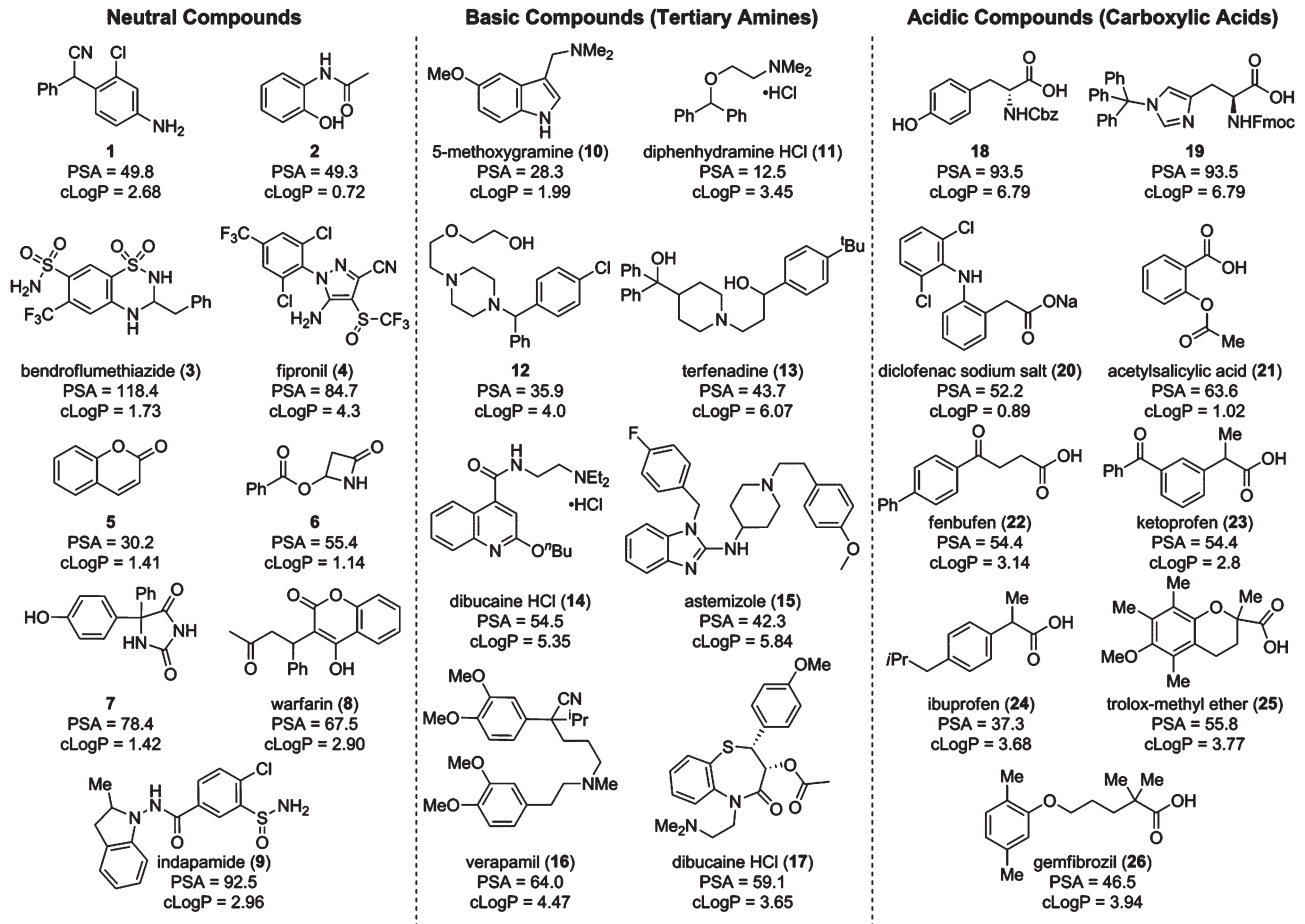

Fig. 1 Neutral, basic and acidic compound sets.

organic chemists seeking to quickly identify greener reaction purification conditions. Skill in effective chromatographic separation is a combination of experience, intuition and experimentation with each mixture to be separated. Thus, the green solvent selection guide is not intended to provide a quantitative measure of eluting strength for any solvent system with any specific compound. Instead, the primary use of this guide should be to rapidly identify starting points to aid chemists in selecting alternative chromatography solvents to DCM while minimizing required experimentation. This guide is intended to supplement the intuition that chemists have for purification and to encourage the use of greener solvent alternatives that may not have been otherwise considered.

This guide was developed using empirical data, and several factors were taken into consideration. First, various solvent mixtures were compared using thin-layer chromatography (TLC), since this is the primary technique employed to select chromatography solvents. Second, the molecules selected for this study were chosen to fall within defined "drug-like" ranges, reflecting the types of molecules that medicinal chemists regularly prepare and purify. Finally, solvent alternatives were selected from previously described lists, and only solvents that are generally considered to be greener alternatives to dichloromethane were evaluated.[3]

Methods

Compound and solvent selection

A selection of commercially available "drug-like" molecules was chosen for this study (Fig. 1).[7] To ensure that the guide would be as broadly useful as possible, a diverse compound set was intentionally selected so that various solvents could be compared without specific molecular attributes biasing the results. Selected molecules display a wide range of functional groups, as well as a range of hydrogen bond donors (0–4) and acceptors (1–6), lipophilicities (cLog P = 0.72–6.07) and polar surface areas (PSA = 12.5–118 Å^2).[8] Finally, compounds were required to exhibit UV absorption at λ = 254 nm to facilitate visualization on TLC plates.

Molecules were then sorted into three subsets: neutral, basic and acidic, each containing 8–9 compounds. Compounds in the neutral subset do not contain a carboxylic acid or an aliphatic amine. The "neutral" designation was liberally applied to this category, and for this application encompasses compounds that we determined would not require acidic or basic additives to avoid tailing or streaking during elution in the solvent systems analyzed. Compounds in the basic subset contain tertiary aliphatic amines. In this case, a basic solvent additive (NH_4OH) was necessary to prevent tailing. Finally, compounds in the

acidic subset contain carboxylic acids, and an acidic solvent additive (AcOH) was necessary to prevent tailing in this case. Ultimately, the laboratory chemist must judge whether or not to use solvent additives depending on the TLC properties of the specific chemical mixture requiring separation.

Chromatography solvents were primarily evaluated using published, well-accepted green solvent selection guides, and those solvents generally regarded as greener than DCM were chosen for this study.[3] The overall greenness was considered, and no single factor (*i.e.* safety, toxicity, environmental impact, *etc.*) was weighted more than another. A diverse set of commonly available solvents was selected to give a range of purification options, a full list of the solvent mixtures tested can be found in the ESI.† Theoretical assessments of the physical properties of these solvents can be found elsewhere.[5] Solvent mixtures were required to be miscible and solvents were required to lack UV activity at $\lambda = 254$ nm. Certain solvent mixtures were also chosen for their feasibility to be stored as stable blends, in particular a 3 : 1 mixture of ethyl acetate and ethanol (EtOAc : EtOH)[9] and a 10 : 1 mixture of methanol and ammonium hydroxide (MeOH : NH_4OH) proved useful in this study.[10] For compounds requiring a basic additive, NH_4OH was incorporated as an additive in the polar solvent phase.[11,12] Similarly, when an acidic additive was required, AcOH was incorporated directly into the polar solvent phase.[13]

Green solvent evaluation method

The relative eluting strength of a particular solvent mixture was determined by TLC analysis of the test compounds.[14] To do this, all compounds within a subset 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.[15] 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. The resulting Rf_{avg} values were plotted against solvent concentration and linear regression lines were fitted to the data (Fig. 2). These regression lines were used to compare the relative eluting abilities of each solvent system.

Fig. 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 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 Rf_{avg} 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. Using this method, the three compound sets were analyzed in various green solvent mixtures.[16] The experimentally-derived relative eluting strengths of different solvent mixtures were then displayed in a bar graph format that is intended to be an accessible reference guide for chemists looking for replacements for DCM in chromatography (Fig. 4).

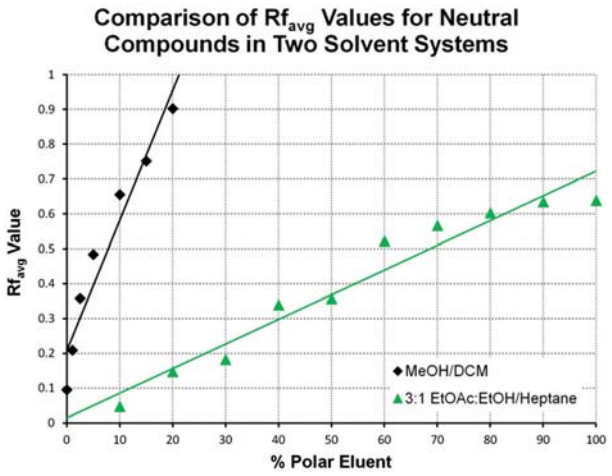

Fig. 2 Data points represent the average Rf (Rf_{avg}) generated from testing the neutral compound set in two solvent mixtures at increasing concentrations of polar eluent. Linear regression lines fitted to the data can be used to compare relative solvent eluting strength at different solvent concentrations.

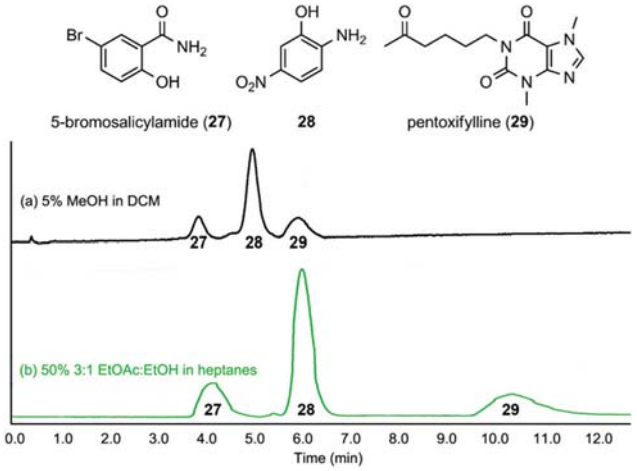

Fig. 3 UV traces showing the separation of a mixture of compounds **27**, **28**, and **29** in two solvent systems: (a) isocratic 5% MeOH in DCM and (b) isocratic 50% 3 : 1 EtOAc : EtOH in heptanes.[17,18]

Method validation

To determine if the empirically-derived green chromatography solvent selection guide is relevant to purification of compounds outside the test set, a mixture of three different compounds (**27**–**29**) was separated by silica gel chromatography using two solvent mixtures.[17] The compounds were selected primarily based on their ability to demonstrate close separation by TLC analysis in 5% MeOH in DCM. The green chromatography solvent selection guide (Fig. 4) was then consulted for an alternative solvent mixture, and 60% 3 : 1 EtOAc : EtOH in heptanes was selected as a starting point for TLC analysis. Quick TLC optimization revealed that a mixture of 50% 3 : 1 EtOAc : EtOH in heptanes would provide optimal separation.[18]

Fig. 3 shows UV traces generated from the chromatographic separation of compounds **27**–**29** in the two solvent mixtures. The

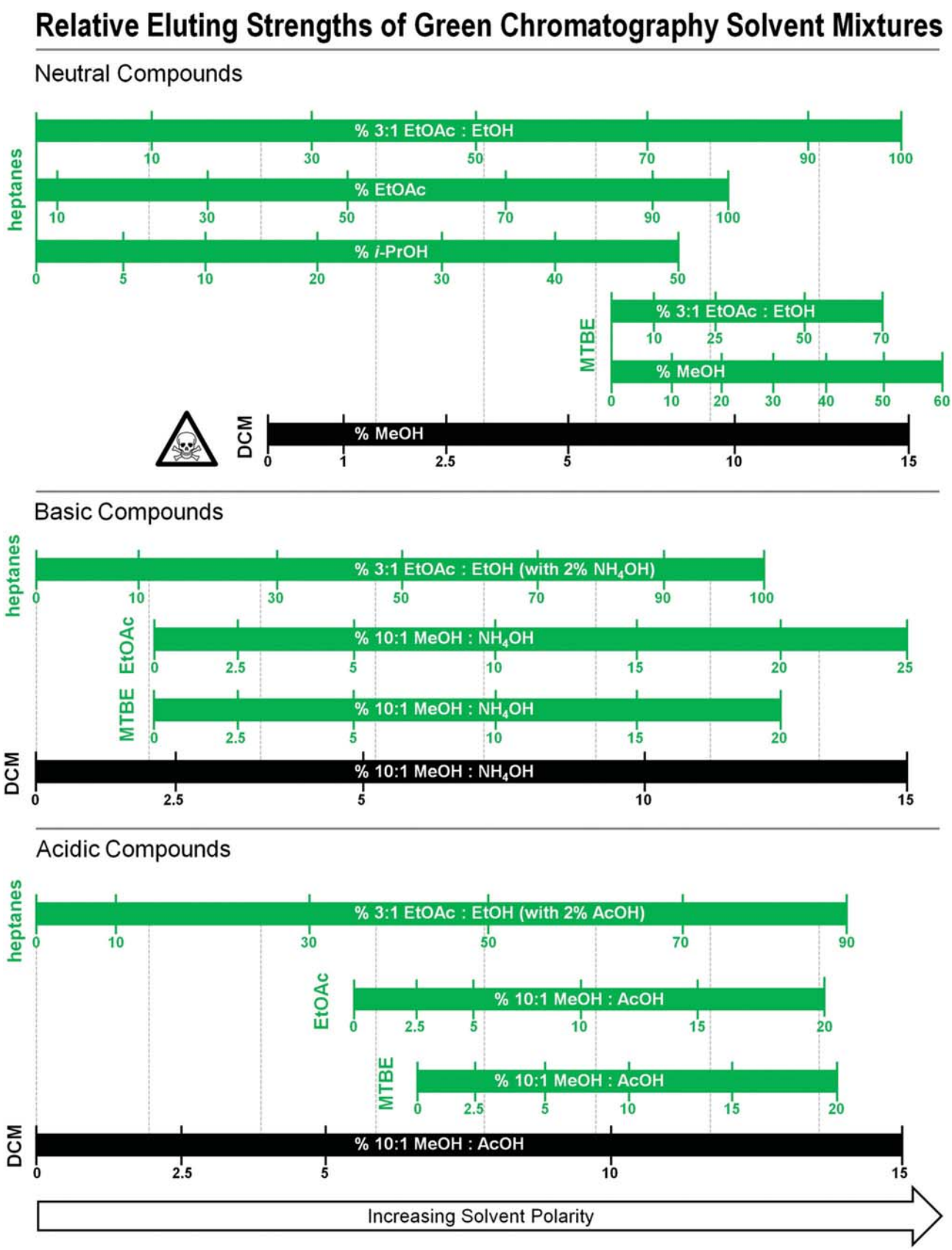

Fig. 4 Green Chromatography Solvent Selection Guide. Starting from the appropriate DCM–MeOH concentration, compare vertically across the bar chart to identify greener solvent mixtures of similar eluting ability. For example, if a compound suitably elutes in 5% DCM–MeOH in the absence of an additive, the "Neutral Compounds" bar chart predicts that 60% 3 : 1 EtOAc : EtOH in heptanes or 40% i-PrOH in heptanes would be suitable starting points to evaluate greener solvent alternatives.

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.

Discussion

Despite the prevalence of DCM use in benchtop chromatography, this solvent can often be satisfactorily replaced with greener solvent alternatives. In our experience, however, medicinal chemists primarily utilize DCM as a chromatography solvent without first considering greener alternatives. The green solvent selection guide presented herein provides medicinal chemists a ready solution to aid choosing a suitable replacement solvent for DCM with minimal expended effort and time. The guide is a focused and convenient aid for specific evaluation and selection of practical and effective alternatives to DCM for chromatography. Using the guide, reasonable concentration starting points are readily found by comparing vertically across the different solvent mixture bars on the chart. For example, if a reaction mixture containing a neutral compound that suitably elutes using a 5% MeOH in DCM solvent system, a quick glance at the green solvent selection guide (Fig. 4) suggests that a 60% 3 : 1 EtOAc : EtOH in heptanes system or a 40% i-PrOH in heptanes system would be good starting points for TLC evaluation with greener solvents. For a more polar neutral compound that suitably elutes in 15% MeOH in DCM, a MeOH in MTBE solvent system could be evaluated. For the bench chemist, empirically deriving these starting points could take significant experimental effort.

TLC evaluation of crude reaction mixtures with alternative green solvent systems is an easy first step toward reduction of DCM in chromatography. Not surprisingly, we have observed that medicinal chemists who regularly perform TLC evaluation of reaction mixtures using green solvents tend to use these solvents more frequently than chemists who do not. Thus, the green solvent selection guide is a useful tool to help overcome the barriers to adoption of green solvents in chromatography and can ultimately help to change the culture of DCM use in the chemistry lab. With this in mind, we have several recommendations for using the green solvent selection guide to best encourage the everyday use of green solvents in chromatography.

• *Clearly display the solvent selection guide* (Fig. 4). This is best done in visible locations on benchtops, near chromatography areas, or near solvent storage areas. A visible guide serves as a continual reminder to consider green chromatography solvents.

• *Stock green solvent blends.* Reducing the amount of solvents that an individual must mix together reduces another barrier to adopting green solvents in chromatography since many chemists are accustomed to using binary solvent mixtures and many automated chromatography systems are configured to use binary solvent mixtures. Because of their stability and broad dynamic range, we suggest stocking the polar eluent blends 3 : 1 EtOAc : EtOH[19] and 10 : 1 MeOH : NH_4OH. Both of these can be combined with green non-polar eluents for use in a binary gradient purification system. However, active discussions with chemists should take place to determine the appropriate blends for any particular department or lab.

• *Actively promote green solvent use.* Continually remind chemists to perform TLC evaluations using alternative green solvent systems when considering DCM for chromatographic purifications. Announcements at departmental meetings, emails, postings, and informal conversations are potential forums to promote green solvent use. Publicize useful examples where green solvents were suitable replacements for DCM.

• *Measure solvent use.* Although DCM use within a department will ebb and flow over time depending on project requirements, long-term measurement of both DCM and green solvent use provides useful insights into how well green solvents are being adopted for chromatography. Measuring and publicizing these data will show the real outcome of choosing alternative green solvents. This can provide positive reinforcement when green solvent usage is high, or this can serve as another reminder to try alternative green solvents if adoption has been lower than expected.

By familiarizing chemists with effective and practical alternative solvent systems, this green chromatography solvent selection tool can facilitate green chemistry efforts by helping chemists to move out of their established DCM comfort zone.

Summary

Incorporating green chemistry practices into medicinal chemistry is a challenging but worthwhile effort. The dynamic nature of this research requires timely adaptability to the wide range of reactions and scales encountered in any given day. Because of this, chromatography will likely continue to be a heavily utilized purification technique and reducing DCM use in chromatography can be a generally applicable green chemistry practice that provides significant positive environmental impact with no loss of research efficiency. To facilitate this endeavor, we have described an empirically-derived green solvent selection guide as a simple tool to encourage chemists to consider alternative solvents to DCM. By providing simple starting points for solvent mixture evaluation this practical guide can help chemists who are engaged in high diversity synthetic chemistry overcome barriers to evaluating green chromatography solvents. Additionally the guide can serve as a visual aid to encourage a scientific culture that considers alternative green solvents on a daily basis.

Notes and references

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8 Calculated using the Daylight DayProp package: http://www.daylight.com/products/dayprop.html

9 For miscibility reasons, EtOH is superior to MeOH as a polar solvent when used in mixtures with heptanes. While we believe that any grade of absolute EtOH will be suitable for chromatography, we used 200 proof EtOH supplied by Decon Labs, Inc.

10 The subject of silica gel stability at high concentrations of MeOH appears to be an issue of significant debate. The following reference indicates that silica gel is stable in alcohol solvents at neutral pH, but decomposes in highly basic water. To be safe, we recommend that MeOH loading be limited to 25% in the presence of NH_4OH, unless stabilized silica gel is used. See: G. B. Alexander, W. M. Heston and R. K. Iler, *J. Phys. Chem.*, 1954, **58**, 453.

11 Concentrated aqueous NH_4OH (JT Baker, 28–30% NH_3) was used. In 3 : 1 EtOAc : EtOH, the concentration of NH_4OH was 2%, while in MeOH the concentration of NH_4OH was 10%. This normalized the amount of NH_4OH throughout all solvent mixtures since 3 : 1 EtOAc : EtOH was evaluated as a polar phase at concentrations as high as 100% while MeOH was evaluated as a polar phase only at concentrations as high as 20%.

12 We recommend mixing the 2% NH_4OH in 3 : 1 EtOAc : EtOH fresh before use, since long-term storage of this blend could lead to trace amounts of acetamide (see ref. 20). To prepare a 4 L bottle of 3 : 1 EtOAc : EtOH with 2% NH_4OH, combine 3 L EtOAc, 1 L EtOH and 82 mL of concentrated NH_4OH. The same method can be applied to the blend of AcOH with 3 : 1 EtOAc : EtOH.

13 Glacial AcOH was used (2% in 3 : 1 EtOAc : EtOH and 10% in MeOH). See. ref. 11.

14 See ESI† for full experimental details.

15 Compounds were visualized under UV light (254 nm).

16 The full Rf_{avg} *vs*. solvent concentration plots for all three compound sets in all solvent mixtures can be found in the ESI.†

17 The mixture contained 27 (10 mg), 28 (2 mg), and 29 (11 mg). Less of compound 28 was employed in the mixture because of the high UV absorbance at $\lambda = 254$ nm.

18 Solvent concentrations were selected so that compound 28 eluted with Rf = 0.2 by TLC analysis in both solvents mixtures. Chromatography was performed on an ISCO Combiflash Rf machine using a RediSep® Rf Gold 4 gram pre-packed SiO_2 gel column. The compound mixture was dissolved in DCM (0.5 mL), loaded on the pre-solvated column via syringe and eluted with an isocratic solvent mixture at a rate of 18 mL min^{-1} for 15 min. UV absorbance was monitored at $\lambda = 254$ nm.

19 3 : 1 EtOAc : EtOH blends can be purchased from both Burdick and Jackson and from Sigma.

20 In our hands, formation of acetamide through the combination of EtOAc and NH_4OH was not observed. A report of combining EtOAc with equimolar NH_4OH revealed that the mixture requires standing for several days to form acetamide. See: I. K. Phelps and M. A. Phelps, *Am. J. Sci.*, 1908, **24**, 429.