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Development of a Tripartite Solvent Blend for Sustainable Chromatography

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A three-component solvent system (isopropyl acetate, methanol, and heptane) was designed to empower routine chromatographic separation of a wide range of compounds, from polar to nonpolar molecules, without the need for undesired chlorinated solvent. This system was evaluated with a pharmaceutically-relevant set of compounds via practical silica gel chromatographic purification, making the solvent blend an attractive sustainable chromatography solution for drug discovery efforts.

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Introduction

Increasing operational sustainability has been a key driver for pharmaceutical companies over the recent past as they seek to achieve greater overall efficiency.[1] With organic solvents the largest contributor to hazardous waste, comprising approximately half of the total material used to manufacture drug substances, green chemistry principles and practices are instrumental in achieving the necessary improvements.[2] Process Mass Intensity (PMI) evaluations of small molecule pharmaceutical syntheses have demonstrated that a decrease in overall solvent use during development has helped reduce waste by orders of magnitude, minimizing both cost and environmental impact.[3] Concurrently, employing use of greener solvents is beneficial as it obviates the use of hazardous substances.

Silica gel chromatography remains a powerful means to purify highly complex organic compounds, particularly in the medicinal chemistry and academic space.[4] In recent years, several commercially-available, automated flash chromatography systems have rendered this practice routine whilst making time-intensive purification more efficient and economical. Nonetheless, the amount of solvent (and other) waste generated by flash chromatography operations is tremendous and inherently unsustainable.[5] Laudable efforts have been made to reuse the pre-packed columns and fraction tubes or recycle silica gel and mobile phase components. A modification in eluent composition makes the procedure more sustainable by utilizing greener solvents, an improvement enhanced by facile implementation.

Two mobile phase blends, composed of four solvents, are traditionally utilized based on the polarity of the compound to be isolated – dichloromethane / methanol (DCM / MeOH, for polar molecules) and hexanes / ethyl acetate (Hex / EtOAc, for nonpolar structures). With chlorinated solvent waste identified as a particularly troublesome subset of organic waste streams, several promising protocols have been published to remove DCM from chromatography. These include eluents incorporating CPME, EtOAc/EtOH, and acetone.[6] In addition, many organizations, including our own, have supplanted hexanes in favour of heptane, which is safer and avoids toxicity and safety issues.[7]

With MeOH and heptane being readily available green chromatography solvents,[8] we sought to identify a third green solvent that would enable a continuous gradient so that purification of the majority of investigational compounds from polar to non-polar (acidic, basic, and neutral molecules) would be possible. While the common solvent EtOAc has some promise for this purpose, it is also susceptible to transesterification in the presence of MeOH.[9] In contrast, it is well known in process chemistry circles that isopropyl acetate (*i*-PrOAc) is a more efficient ester solvent in extractive work-up procedures due to its reduced aqueous solubility and hydrolytic lability (vs. EtOAc). Transesterification is similarly reduced due to the branched alkyl group. Based on this robustness, we set out to investigate the potential of *i*-PrOAc to serve as the bridging solvent in our desired tripartite chromatography eluent.

In this report, we establish that a solvent system based on these three components (*i*-PrOAc, MeOH, and heptane, plus acidic and basic modifiers) can be used to achieve the separation of a great variety of compounds and could be implemented to target the bulk of all silica gel chromatographic purifications within a drug discovery organization. In addition to providing a wide-ranging elution matrix for molecules extending from polar to non-polar nature, the three green solvents would help streamline bulk solvent management / procurement and minimize worker safety concerns.

Results and Discussion

In order to fully evaluate the separation potential for *i*-PrOAc / MeOH / heptane across a diverse range of pharmaceutically relevant compounds, we created a compound set with representative drug and drug-like molecules (Figures 1a through 1c). The collection was designed to include approximately thirty compounds of acidic, basic, and neutral composition. To further ensure applicability, the compound set covered a range of pharmaceutically desirable properties,[10] varying in 1) number of hydrogen bond donors, 2) number of hydrogen bond acceptors, 3) molecular weight, 4) number of rotatable bonds and 5) topological polar surface area.

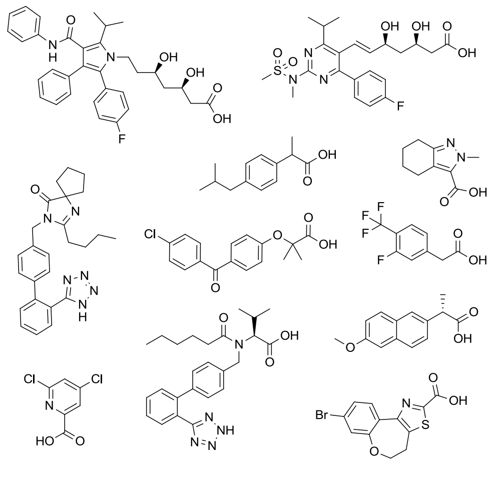

Figure 1a. Acidic molecules in compound set (11 molecules).

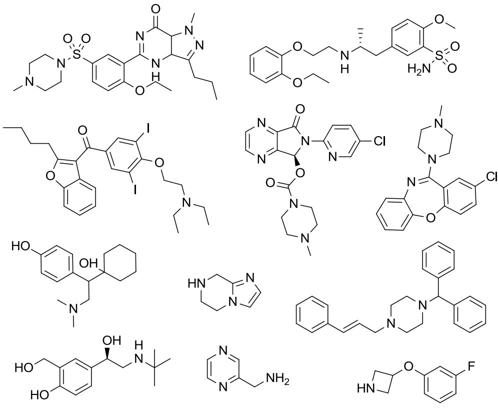

Figure 1b. Basic molecules in compound set (11 molecules).

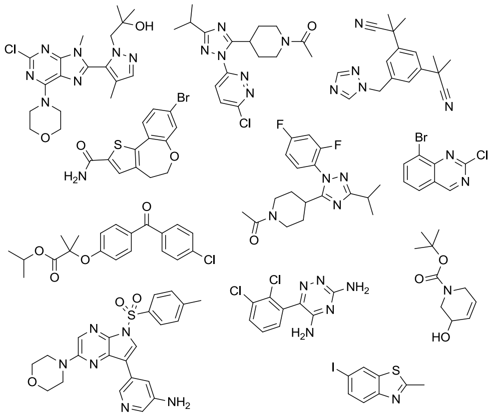

Figure 1c. Neutral molecules in compound set (11 molecules).

To determine the best proportion of solvent composition in the proposed ternary system (and establish a robust method to accurately meter in low levels of the polar component methanol), we evaluated several fixed ratios of *i*-PrOAc : MeOH solutions (6:1, 5:1, 4:1, and 3:1) and ran them with heptane – isocratic for thin layer chromatography (TLC) and in a gradient for columns. Of these binary solvent mixtures, the 3:1 ratio consistently provided superior separation and served as the foundation for our remaining investigation. In order to establish confidence in the stability of these solutions, we examined them by headspace gas chromatography (GC) over several time points.[11]

The first evidence of the separation capability of the tripartite solvent system was demonstrated by TLC. Each of the 33 compounds was tested in 0, 1, 3, 5, 10, and 20% MeOH in DCM as well as in 10%-increments from 0 to 100% 3:1 *i*-PrOAc : MeOH in heptane. A basic (ammonium hydroxide or triethylamine) or acidic (acetic acid) additive was included for basic and acidic compounds, respectively. The retardation factors (R_f) of each compound in the various eluent systems were calculated and the relationship between percentage polar eluent and the corresponding R_f value plotted. The two graphs for each compound (one for each of the two solvent systems) were superimposed, to compare the percentage equivalencies between the two solvent systems that generated the same R_f values. From these overlays, three resultant plots (one each for acidic, basic and neutral compounds, Figures 2a through 2c) were furnished which represent the averaged equivalency values (with one standard deviation above and below) of the individual compound graphs. An additional graph was made combining the data points for the three separate plots (Figure 2d).[12]

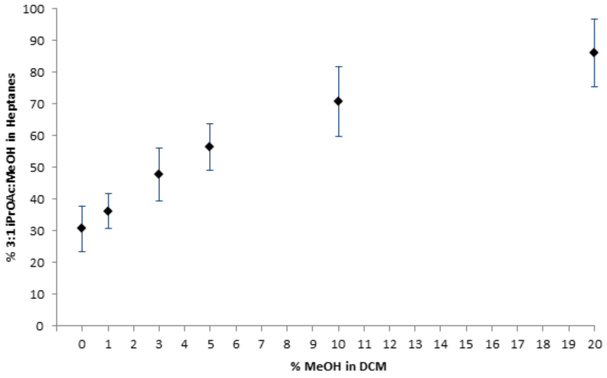

Figure 2a. Graph depicting corresponding polarity of solvent mixtures based on equivalent R_f values of a set of 10 acidic compounds with 2% acetic acid as additive. Error bars correspond to one standard deviation.

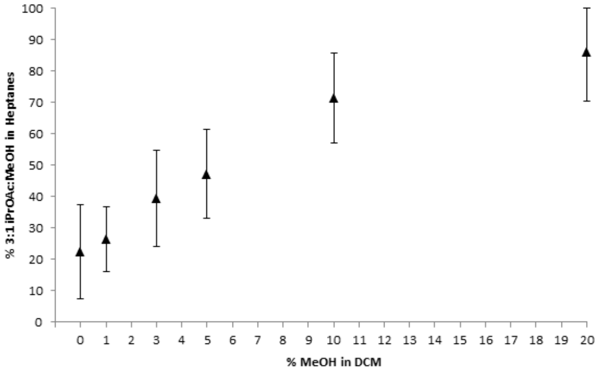

Figure 2b. Graph depicting corresponding polarity of solvent mixtures based on equivalent R_f values of a set of 10 basic compounds with 2% ammonium hydroxide as additive. Error bars correspond to one standard deviation.

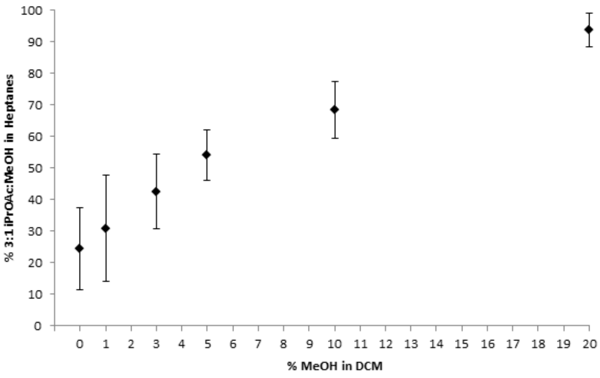

Figure 2c. Graph depicting corresponding polarity of solvent mixtures based on equivalent R_f values of a set of 10 neutral compounds. Error bars correspond to one standard deviation.

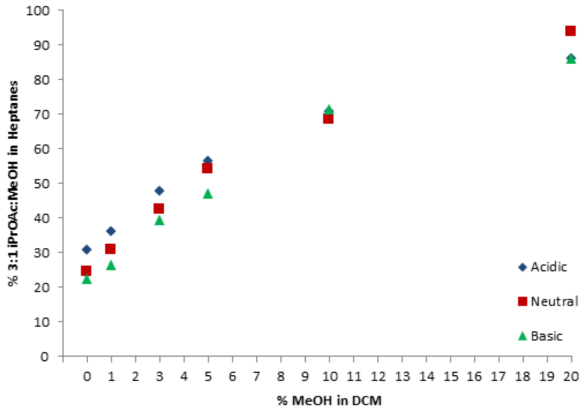

Figure 2d. Graph depicting corresponding polarity of solvent mixtures based on equivalent R_f values of a set of representative compounds.

After TLC analysis, the 3:1 *i*-PrOAc : MeOH in heptane solvent system was tested with an automated Teledyne Isco Combiflash® chromatography system to determine whether the separation of compounds could be successfully translated to actual column chromatographic purifications.[13] For purposes of direct comparison, mixtures of three drug-like compounds were separated on the Isco system in both the traditional MeOH / DCM eluent and the 3:1 *i*-PrOAc : MeOH / heptane mobile phase with triethylamine as an additive (Figures 3a and 3b).

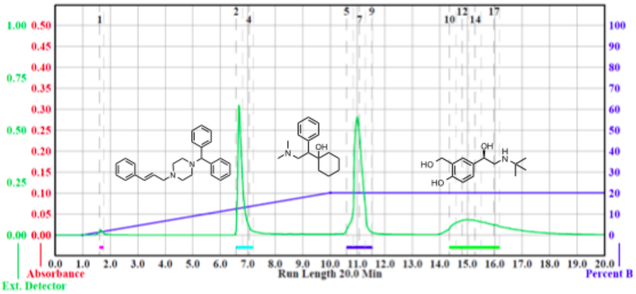

Figure 3a. Chromatogram resulting from separation of three basic compounds with a 0-20% gradient of MeOH in DCM.

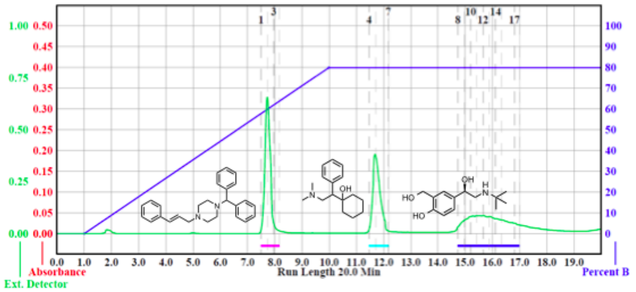

Figure 3b. Chromatogram from separation of three basic compounds with 0-80% gradient 3:1 *i*-PrOAc : MeOH in heptane.

For each run, the solvents were introduced in gradient fashion: 0-20% MeOH in DCM and 0-80% 3:1 *i*-PrOAc : MeOH in heptane. In each case, the percentage of total MeOH was maintained $\leq$20% to avoid any risk of dissolution of the silica solid phase. In brief, the two solvent systems showed comparable separation for the compound mixtures, confirming that the results from TLC could be translated to practical chromatographic separation.

The translation to practical silica gel separation demonstrates the applied utility of this study and complements the other recent sustainable chromatography reports. Our method utilizes solvents that are readily available in bulk format and have a low propensity for cross- or self-reactivity. Thus the *i*-PrOAc / MeOH / heptane eluent system should enable facile purification of various compound mixtures with few barriers to implementation.

To ease adoption of the proposed greener solvent system, Figures 2a-d should aid chemists accustomed to using MeOH / DCM. The graphs show what percentage of 3:1 *i*-PrOAc : MeOH / heptane provides equivalent TLC separation to the MeOH / DCM result. Figures 3a and b demonstrate that 3:1 *i*-PrOAc : MeOH / heptane provides similar chromatographic separation to the traditional system, with little difference in elution time when the appropriate gradient is selected.

One valuable observation made during the course of this work is that controlled introduction of the highly polar solvent MeOH as a solution in *i*-PrOAc appears to limit the "solvent front effect" that often occurs with MeOH. Dilution of MeOH with *i*-PrOAc also enables better separation between closely spaced peaks as higher %-levels of 3:1 *i*-PrOAc : MeOH are necessary to achieve the equivalent separation witnessed with DCM / MeOH.

A final point on the merits of the 3:1 *i*-PrOAc : MeOH / heptane solvent system is related to costs of raw materials and costs of waste disposal. Based on evaluation of our internal metrics with solvent volumes and run times similar between the two solvent systems as illustrated in Figures 3a and b, the benefit of switching away from DCM / MeOH is a relative factor of 1.91.[14] Thus, it costs roughly twice as much to use the non-green solvent system.

The promising results from our work indicate that the routine practice of chromatography can be conducted in a much greener format. Switching from traditional, but unsustainable, solvent systems to *i*-PrOAc / MeOH / heptane requires minimal effort and yet has immediate improvement on both operator exposure and environment footprint.

Conclusions

Silica gel chromatography remains a widely used and powerful method to purify compounds in the discovery space. Due to heavy solvent utilization, particularly involving DCM, the practice leaves a large environmental footprint. While alternative solvent systems have been described, this paper guides chemists to a robust tripartite solvent mixture that provides separation comparable with traditional eluents. The *i*-PrOAc / MeOH / heptane method has been demonstrated in multiple chromatographic purifications with a direct comparison between the traditional and greener mobile phases. The proposed solvent system should have positive influence on solvent sourcing, worker safety, hazardous waste disposal costs, and overall environmental impact.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [general experimental procedure and spectroscopic data]. See DOI: 10.1039/b000000x/

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11 Eight *i*-PrOAc / MeOH sample solutions with acidic, basic or no additives showed excellent stability up to 60 days, without any noticeable transesterification or other degradation. See Supporting Information.

12 Further illustration of the utility of these graphical data as well as the full data set is provided in the Supporting Information.

13 http://www.isco.com/products/products2.asp?PL=10110&image=Chromatography3.jpg

14 The cost factor was determined by dividing our internal relative disposal cost for halogenated vs. non-halogenated waste by the relative costs of solvents in the two runs represented by Figures 3a and 3b. Please see the Supplementary Information for further detail.

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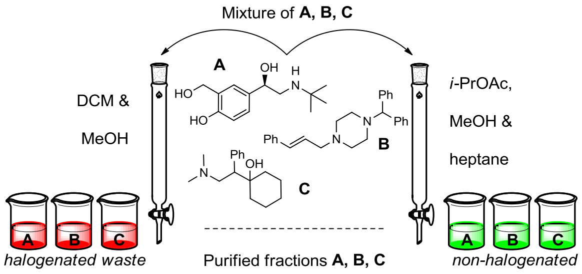

Favorable solvents supplant dichloromethane in routine silica gel chromatography and eliminate halogenated waste, thereby improving safety, economics, and environmental impact.