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Safer Solvents for Active Pharmaceutical Ingredient Purification Using Column Chromatography

Christian Ayafor, Toren Burton, Nathaniel George, Gregory Morose, and Hsi-Wu Wong*

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ABSTRACT: Column chromatography is a technique widely used for the purification of active pharmaceutical ingredients (APIs). One of the common solvent systems used by this technique is blends of dichloromethane (DCM) and methanol (MeOH), thereby exposing workers to health and safety risks and making the pharmaceutical sector one of the major contributors to chlorinated solvent waste. In this work, API separation and purification using several alternative safer solvent blends in column chromatography were evaluated and compared to DCM/MeOH. Ibuprofen and acetaminophen were used as model APIs, and caffeine was used as a model additive. Overall, some of the safer solvent blends tested provided better performance, with higher API recovery and purity compared to DCM/MeOH, in addition to

potential health, safety, and environmental benefits. Specifically, blends of heptane/ethyl acetate and heptane/methyl acetate showed the most promise. Our work demonstrates the potential of these safer solvent blends as possible replacements for DCM/MeOH in API purification, thereby addressing a critical safety concern in the pharmaceutical industry.

KEYWORDS: safer solvents, active pharmaceutical ingredients, purification, column chromatography, thin-layer chromatography
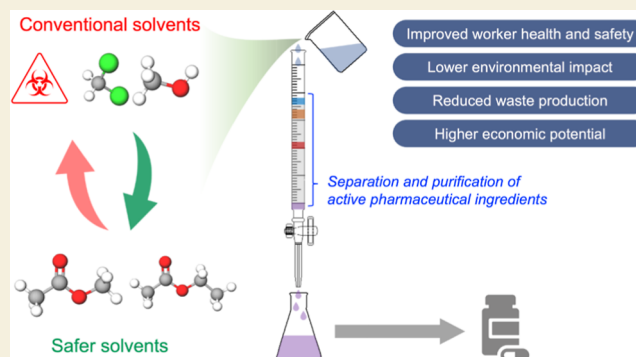
1. INTRODUCTION

Dichloromethane (DCM) is extensively used as a solvent for a wide array of applications such as biopharmaceutical manufacturing, metal cleaning, paint stripping, aerosol propelling, and decaffeination in the food industry.[1,2] A significant amount of DCM is produced annually in the United States, including approximately 120,000 MT reported in 2016.[2,3] In 2021, close to 862 MT of DCM were utilized in various manufacturing processes in Massachusetts alone, with over a third generated as a byproduct and an estimated 18 MT released into the environment.[4]

The U.S. Department of Health and Human Services and the U.S. Environmental Protection Agency (EPA) have associated DCM with a variety of health problems, including cancer and damage to the central nervous system.[1,2] DCM also endures in the environment, with a half-life in water of more than 18 months.[5] Researchers have requested for a re-evaluation of the United States' existing regulatory strategies of paint strippers and other occupational solvents containing DCM due to the continuous occurrence of fatalities and other serious injuries.[6] More recently, the Biden administration instructed the EPA to review its procedures for evaluating chemical risks under the 2016 revisions to the Toxic Substances Control Act.[7] Following this review, the EPA found unreasonable risk of DCM to workers, occupational non-users, consumers, and bystanders.[8] Three hazardous

chemical rating systems: the GreenScreen,[9−11] the GlaxoSmithKline (GSK) solvent selection guide,[12−14] and the Pollution Prevention Options Analysis System (P2OASys),[15,16] all have designated DCM as a high hazard substance. Specifically, DCM has a GreenScreen Benchmark score of 1 (i.e., BM-1, which is a chemical of high concern that should be avoided), a GSK rating of 4 (on a scale of 1 to 10, where 1 is of highest concern), and a P2OASys rating of 7.9 (on a scale of 2 to 10, where 10 is of highest concern). Replacing DCM with a safer solvent or a safer solvent blend will provide significant environmental, health, and safety benefits.[17−25] This goal, however, has remained a hurdle over the past several decades.[26] The major challenge is the performance and cost of the alternatives that cannot be compromised.[17]

Solvents used in the pharmaceutical industry typically account for significant life cycle impacts, as 80−90% of the total mass used in active pharmaceutical ingredient (API) production is attributed to solvents, which are commonly
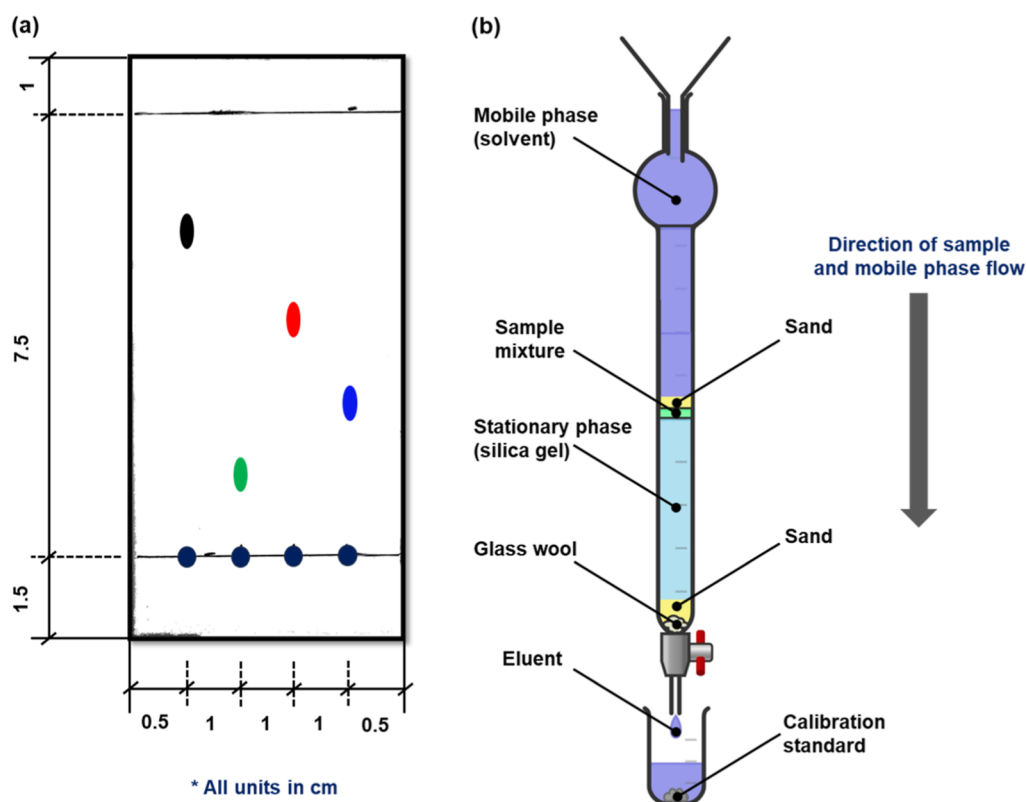
Figure 1. Schematic of the (a) TLC plate and (b) lab-scale column chromatography employed in this work.

disposed of as waste.[27,28] DCM is one of the most commonly used solvents for API purification via column chromatography, making it one of the major contributors to chlorinated solvent waste.[29] The pharmaceutical companies in Massachusetts, for example, consumed more than a third (close to 227 MT) of the total DCM used in the state.[4] There exist a few studies investigating potential replacements for DCM in chromatographic applications. Taygerly et al.[29] studied safer solvent alternatives to DCM in chromatography based on safety, environmental impact, toxicity, elution strength, and other factors. Several safer solvent blends, pairing one of heptane, ethyl acetate, or methyl tert−butyl ether with another more polar solvent from methanol (MeOH), ethanol, iso-propanol, or ethyl acetate (or their mixtures), were identified as potential replacements for the DCM/MeOH blend. The thin-layer chromatography (TLC) retention factors (R_f) of a set of "drug-like" compounds dissolved in different solvent blend systems were measured. A green chromatography solvent selection guide was developed based on the TLC R_f values to help quickly determine the types and compositions of the potentially safer solvent blends in lieu of DCM. The proposed solvent selection strategy was, however, only validated by qualitative separation of the compounds in silica gel chromatography. A later study by MacMillan et al.[30] took a similar TLC approach to investigate several alternative solvents as potential replacements for the DCM/MeOH blend in API purification. Cyclopentyl methyl ether was suggested as the solvent with the most potential, with dimethylcarbonate and ethyl acetate as the other two solvents showing promises. Recently, Sharma et al.[31] developed a methodology that utilizes Hansen Solubility Parameter in Practice (HSPiP),[32] a powerful software tool that has demonstrated to provide fast estimates of solvent and solvent blend properties,[9,33,34] to identify alternatives to DCM for API purification via column chromatography. Several safer solvents and their blends were proposed based on the solution properties estimated by HSPiP, safety ratings, and price. TLC experiments were conducted to test the performance of these safer solvents and blends in separating one of the three commonly used APIs (ibuprofen, acetaminophen, and aspirin)[35,36] from caffeine, the model additive.

Investigations into the quantitative performance of these safer solvent blends to replace DCM in actual column chromatography, such as recovery ratio and purity of the APIs, have not been undertaken.[19−21] The goal of the present study is to quantitatively determine the performance of different solvent blends for replacing the DCM/MeOH blend in actual column chromatography, taking heptane as the nonpolar solvent, suggested by Taygerly et al.,[29] and blending heptane with one of the polar safer solvents identified by Sharma et al.[31] Note that heptane has a GreenScreen rating of BM-2 and a GSK rating of 8, significantly better than DCM's rating of BM-1 and 4, respectively. In addition, heptane is less polar than DCM, potentially covering a wider range of polarities when blended with a polar solvent for better solute compatibility. Finally, heptane is a common solvent for alternative chromatography techniques, such as dry column vacuum chromatography,[37] which increases public reception and lowers the commercial hurdle of our proposed formulation. In our work, a lab-scale column chromatography was configured for solvent evaluation. The performance of separating model APIs (ibuprofen and acetaminophen) from a model additive (caffeine) was assessed by the API recovery ratio and purity. The most appropriate alternatives to the DCM/MeOH blend for column chromatography applications are recommended.

2. EXPERIMENTAL METHODS

2.1. Materials

DCM (≥99.8% purity), methyl acetate (≥99% purity), and caffeine (≥99.7% purity) were obtained from Alfa Aesar, Ward Hill, MA, USA. 1,3-Dioxolane (≥99% purity), acetaminophen (≥98% purity), and aspirin (≥99.5% purity) were obtained from Spectrum Chemicals, New Brunswick, NJ, USA. Ethyl acetate (≥99% purity) and ibuprofen (≥99% purity) were obtained from Acros Organics, Geel, Belgium. Acetone (≥99.5% purity) and MeOH (≥99.9% purity) were obtained from Fisher Chemicals, Fair Lawn, NJ, USA. Dimethyl adipate (≥99% purity) was obtained from TCI, Tokyo, Japan. Washed sand and silica gel (0.060−0.2 mm, i.e., 70−230 mesh) were obtained from Alfa Aesar, Heysham, Lancashire, UK. Deactivated glass wool was obtained from Restek, Bellefonte, PA, USA. Phosphoric acid (≥85% purity) and Synthware chromatography columns with standard taper joint, reservoir, and polytetrafluoroethylene stopcock were obtained from Fisher Scientific, USA. TLC plates (Supelco TLC Silica gel 60 F_{254} Plates 20 × 20 cm) and the ultraviolet (UV) torch (Supelco UV lamp 254 nm for TLC) were obtained from Millipore Sigma (Burlington, MA, USA). Glass capillary tubes for dissolution tests were purchased from Fisher Scientific (Waltham, MA, USA). The manual glass cutter (Fletcher-Terry gold tip glass cutter) was acquired from McMaster-Carr (Elmhurst, IL, USA).

2.2. TLC

In this work, ibuprofen and acetaminophen were chosen as model APIs, representing acidic and neutral compounds as defined by Taygerly et al.,[29] respectively. Caffeine was selected as a model additive,[31] since it generally constitutes a significant percentage of these APIs when commercialized. Aspirin was chosen as a calibration standard for the quantitative analysis.

TLC plates were cut into 4 × 10 cm (width × height) pieces. The origin line was marked 1.5 cm from the bottom of each TLC plate, and the solvent front line was marked 1 cm below the top of each TLC plate, allowing a travel distance of 7.5 cm. Four spots were marked 1 cm apart on the origin line with the outer spots 0.5 cm from the left and right flanks of the TLC plate. The solutions spotted on the TLC plate consisted of 3 mg mL^{-1} of each of the analytes (ibuprofen, acetaminophen, caffeine, and aspirin) in acetone. The TLC experiments were conducted using the protocol described in the literature, and the developed plates were observed with a UV lamp at a wavelength of 254 nm.[38−41] A schematic of the TLC plate is shown in Figure 1a.

2.3. Column Chromatography

A schematic of the lab-scale atmospheric column chromatography used in this study is shown in Figure 1b. Silica gel was chosen as the stationary phase in the column to be consistent with the TLC experiments. The wet packing method was used to load the silica gel into the column.[42,43] Specifically, glass wool was first loaded at the bottom of the column to prevent the sand and silica gel from flowing through. Washed sand was placed above the glass wool to provide an even surface on which the silica gel could be loaded. After the sand and glass wool were soaked with the eluent that would be utilized as the mobile phase during the experiments, the stopcock was closed while maintaining a reasonable level of the mobile phase above the sand, endeavoring that a uniform top surface of the sand was not perturbed when silica gel was poured in. Slurry silica gel (a mixture of silica gel and eluent) was then added above the sand layer and allowed to settle for 30 to 45 min, after which a thin layer of sand was added above the silica gel to prevent any displacement of the silica gel surface while conducting the experiments.

Each of the column chromatography experiments was undertaken with 99 mg of a sample mixture, consisting of 33 mg of each model API (ibuprofen or acetaminophen) and 33 mg of the model additive (i.e., caffeine), dissolved into 2 mL or less of a mobile phase used as the eluent contained in a capped 20 mL vial. The capped solutions were then briefly placed on top of a heating plate (Fisherbrand Isotemp HP88857200) maintained at 70 °C without solvent boiling

observed to ensure that all of the solid compounds were dissolved into the solvent prior to being dispensed from the top of the column. The dispensed warm solution into the column was expected to cool, and solid precipitation was possible. However, this was not considered a major issue since all of the solid compound mass was believed to be loaded into the column above the top sand layer in either dissolved or precipitated solid form, allowing for subsequent performance evaluation.

The initial experiments were conducted with a total of 5 g of silica gel contained in the column, achieving a compound/silica gel ratio of approximately 1:50. The amount of silica gel was later increased to 15 g, corresponding to a taller column and a compound/silica gel ratio of approximately 1:150 for compound mixtures that are more challenging to separate. For both silica gel loadings, the initial liquid level of the mobile phase was near the top of the column. Once the stopcock was opened, this liquid level was maintained the same as possible throughout each experiment by pouring in additional solvent. The elution was collected in volumes of 2 mL into borosilicate culture tubes (VWR, 13 × 100 mm) containing 1 mg of aspirin as the calibration standard. The amounts of the APIs and caffeine in each collected sample were then quantified by high-performance liquid chromatography (HPLC).

2.4. Preparation of the Calibration Standards

For the quantification of the APIs in the solutions, four standard solutions in different ratios were prepared for HPLC calibration by dissolving the purchased compounds (i.e., ibuprofen, acetaminophen, and caffeine, with aspirin as the calibration standard) into 10 mL of each mobile phase.[9] Note that the concentrations of these solutions were approximately 18 to 55 times lower than those used to load the column and were more representative to concentrations of the compounds in the eluents. Aspirin was chosen as the calibration standard because it does not overlap with the APIs and caffeine during their quantification on HPLC. The compounds in the standard solutions were loaded as Standard 1 (ibuprofen: 1 mg, acetaminophen: 1 mg, caffeine: 1 mg, and aspirin: 6 mg), Standard 2 (ibuprofen: 3 mg, acetaminophen: 3 mg, caffeine: 3 mg, and aspirin: 6 mg), Standard 3 (ibuprofen: 6 mg, acetaminophen: 6 mg, caffeine: 6 mg, and aspirin: 6 mg), and Standard 4 (ibuprofen: 9 mg, acetaminophen: 9 mg, caffeine: 9 mg, and aspirin: 6 mg). The solutions were again capped and briefly placed on top of a heating plate maintained at 70 °C without solvent boiling observed to ensure that all of the solid compounds were dissolved.

2.5. Product Analysis

The quantification of products was achieved by an Agilent 1100 series HPLC system equipped with a diode array detector (DAD). A Luna C18(2) 100 Å column (150 × 4.6 mm, 5 μm particle size) was used with a column temperature maintained constant at 40 °C. A solution of 20 mM phosphoric acid in deionized water was used as mobile phase A, and MeOH was used as mobile phase B. The system flow rate was maintained by the G1312A binary pumps at 0.6 mL min^{-1}. Each sample was injected by the G1329A autosampler with an injection volume of 5 μL at ambient temperature. The analytes were detected by the G1315A DAD at a wavelength of 265 nm. The HPLC gradient was programmed as follows (time, % of mobile phase A): (1) 0 min, 80% A; (2) 15 min, 35% A; and (3) 20 min, 80% A.

Figure S1a shows the chromatograms of the four standard chemicals in the 87.5 vol % DCM/MeOH blend where good separation of the peaks was observed. The linear calibration of ibuprofen, acetaminophen, and caffeine against aspirin (Figure S1b) confirms that no solid precipitation occurred in our calibration standard solutions, and our analytical method was robust for product quantification.

3. RESULTS AND DISCUSSION

3.1. Solvent Analysis Based on TLC

The chemical hazard ratings, viscosity, cost, and Hansen Solubility Parameter (HSP) values of DCM, MeOH, and

Table 1. Comparison of Chemical Hazard Ratings, Viscosity, Cost, and HSPs of the Solvents[a]

| solvents	CAS #	[b]GreenScreen score	GSK health score	P2OASys score	[c]viscosity at 25 °C (cP)[44,45]	[d]cost ($/L)	[e,f] Hansen solubility parameters (MPa$^{1/2}$)[46-48]			[g]dielectric constant
		BM1 - high hazard BM4 - low hazard	1 - high hazard 10 - low hazard	10 - high hazard 2 - low hazard			δD	δP	δH	ε
dichloromethane (DCM, to be replaced)	75-09-2	BM-1	4	7.9	0.44	110.0	17.0	7.3	7.1	8.93
methanol (MeOH)	67-56-1	BM-1	5	6.6	0.54	91.8	14.7	12.3	22.3	33.00
heptane	142-82-5	BM-2	8	7.8	0.41	205.0	15.3	0.0	0.0	1.92
ethyl acetate	141-78-6	BM-2	8	6.4	0.42	127.0	15.8	5.3	7.2	6.08
cyclohexanone	108-94-1	LT-P1	6	6.6	2.20	61.4	17.8	8.4	5.1	16.10
1,3-dioxolane	646-06-0	BM-2	7	6.1	0.55[a]	149.0	18.1	6.6	9.3	7.13
acetone	67-64-1	BM-2	8	5.4	0.32	125.0	15.5	10.4	7.0	21.01
methyl acetate	79-20-9	BM-2	7	4.1	0.36	97.3	15.5	7.2	7.6	7.07
dimethyl adipate	627-93-0	LT-UNK	no evaluation	3.5	2.50	124.0	16.3	6.8	8.5	6.84
silica	7440-21-3						16.7	13.7	14.0	
ibuprofen	15,687-27-1						17.6	2.5	7.6	
acetaminophen	103-90-2						17.8	10.5	13.9	
caffeine	58-08-2						19.5	10.1	13.0	

[a]The HSPs of the chemical compounds studied in this work and of silica, the column stationary phase, are also listed. [b]The GreenScreen for Safer Chemicals is a framework for characterizing hazardous risks associated with chemicals by analyzing 20 human health and environmental hazard end points. Each chemical will receive a benchmark (BM) score on a 4-point scale from highest to lowest concern (BM-1 to BM-4). BM-1 (of high concern and to be avoided) is reserved for chemicals that are carcinogens, reproductive, developmental and neurodevelopmental toxicants, mutagens, persistent, bioaccumulative, and toxic chemicals (PBTs), very persistent toxicants (vPTs), very bioaccumulative toxicants (vBTs), very persistent, very bioaccumulative substances (vPvBs), and endocrine disruptors. BM-2 chemicals (recommended for use but search for safer substitutes) are those with high hazards for other human health end points, such as neurotoxicity and respiratory sensitization. LT-P1: List Translator Possible Benchmark 1; LT-UNK: List Translator Unknown. [c]Viscosity is based on information from PubChem[44] and Guiochon et al.[45] at 20 °C. [d]Cost information is obtained from the Sigma-Aldrich Web site based on 1 L of the solvent in either HPLC Plus grade or ReagentPlus grade (retrieved on April 27, 2024). [e]HSPs are used to describe solvent and solute interactions based on contributions from three intermolecular force: dispersion forces (δD), polar forces (δP), and hydrogen bond forces (δH). A substance's solubility can be expressed by HSPs as a point in a three-dimensional space using the three parameters.[31,46] [f]HSPs of silica gel are obtained from Fujiwara et al.[48] [g]Dielectric constants are obtained from the 95th edition of the CRC Handbook of Chemistry and Physics,[49] except that for 1,3-dioxolane, which is taken from Gu et al.[50]

heptane, as well as six safer solvents identified by Sharma et al.,[31] are listed in Table 1. Since species separation using column chromatography is largely affected by the polarity of the mobile phase and the compounds, the dielectric constant (ε) for each solvent, a quantitative indicator of solvent polarity,[50] is also listed. The HSP values of the three chemical compounds studied in this work and of silica, the stationary phase materials used in our actual column chromatography experiments, are also included. Among the different solvents, cyclohexanone was not considered in our study due to its high viscosity and safety ratings close to those of MeOH (Table 1), suggesting potentially unsatisfactory performance with heightened hazards of its use in a closed laboratory setting. 1,3-Dioxolane was also dropped due to its high cost compared to those of DCM and MeOH. The remaining safer solvents identified by Sharma et al.[31] all have better GreenScreen and GSK ratings than both DCM and MeOH. The only exception was dimethyl adipate, with no reported GreenScreen or GSK ratings but a better P2OASys rating compared to all the other solvents.

The evaluation of solvent performance was first undertaken using TLC as described in the literature.[29,30] TLC enabled the screening of solvent blends that showed comparable R_f values (the ratio of the distance traveled above the origin line by the analyte spots to that by the solvent fronts) for given analytes. In selecting the optimal solvent blend compositions, the industrially recommended $0.4 \leq R_f \leq 0.8$ was taken as a reference range.[31] For an $R_f < 0.4$, the solvent system is considered too nonpolar, and the compounds could be too slow to elute from the column, while for an $R_f > 0.8$, the solvent system is too polar and the compounds could elute too quickly from the column with poor separation.

For our initial TLC experiments, each of the four analytes (ibuprofen, acetaminophen, caffeine, or aspirin) was dissolved in acetone at a concentration of 3 mg mL^{-1}, and the four solutions were parallelly spotted on a TLC plate and eluted with each solvent blend, which was thoroughly examined with different compositions with increasing solvent polarity before the next solvent pair was studied. For example, for the DCM/MeOH blend, an experiment was initially carried out with pure DCM, which represents the lowest polarity for this solvent system. MeOH was then added in 5 vol % increments; thereby, the polarity of the solvent blend was slowly increased until 25 vol % MeOH in DCM was attained. For heptane blending with one of the safer solvents, the initial experiment was conducted with pure heptane. The polarity of the blends was then increased through the addition of one of the safer polar solvents in 10 vol % increments. Pure safer solvents that did not contain any heptane were also tested. Figure 2 shows the R_f values for every tested heptane/safer solvent blend against DCM/MeOH, with all four analytes plotted in the same graph. The R_f values sorted by each analyte with varying compositions of different heptane/safer solvent blends are provided in Figure S2.
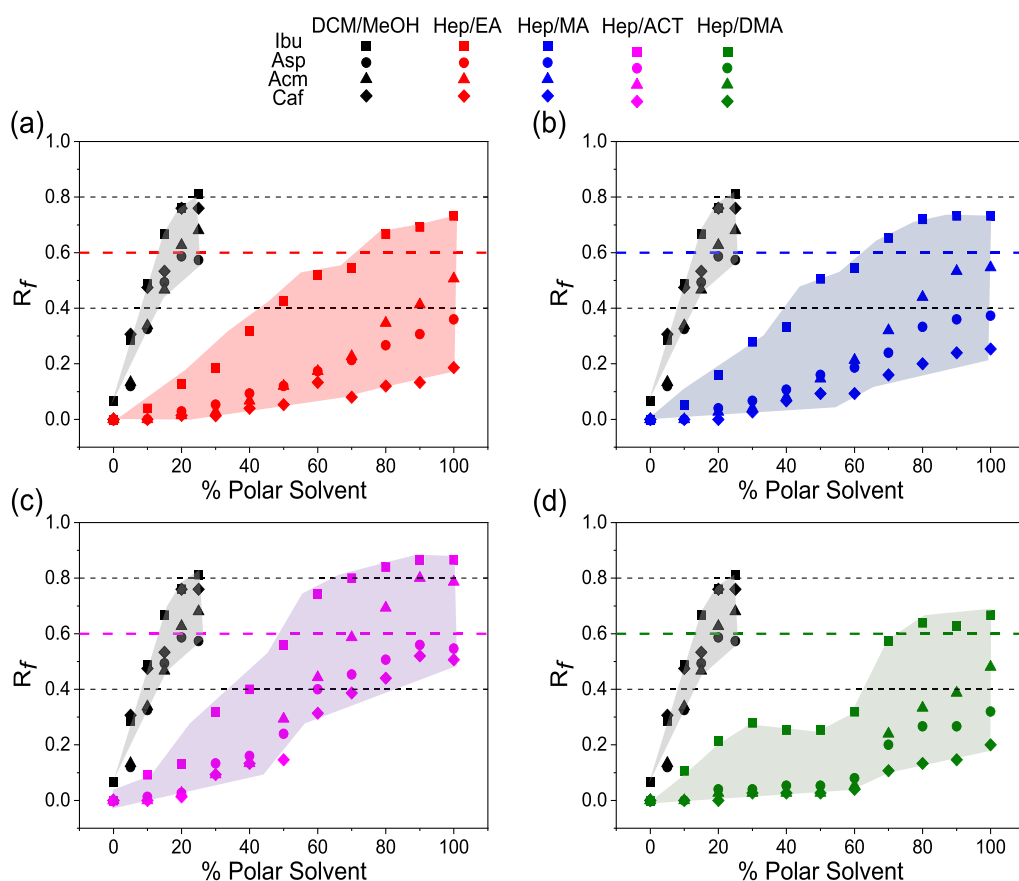
Figure 2. Comparison of R_f values of four different analytes: ibuprofen (Ibu), aspirin (Asp), acetaminophen (Acm), and caffeine (Caf) in DCM/MeOH (shaded black) against (a) heptane/ethyl acetate (Hep/EA, red), (b) heptane/methyl acetate (Hep/MA, blue), (c) heptane/acetone (Hep/ACT, purple), and (d) heptane/dimethyl adipate (Hep/DMA, green).

**Table 2. Selected Compositions of Various Solvent Blends for Further TLC Analysis**

solvent blends (%)							
DCM	MeOH	heptane	ethyl acetate	heptane	methyl acetate	heptane	acetone
90	10	25	75	30	70	35	65
85	15	15	85	20	80	25	75
80	20	5	95	10	90	15	85

The obtained R_f graphs were then used to determine the compositions for each solvent blend for further comparative studies. An R_f of 0.6, which is the center point of the recommended R_f range ($0.4 \leq R_f \leq 0.8$), was selected for this exercise (colored dash lines in Figure 2). Considering all four compounds, an R_f of 0.6 corresponds to approximately 10−20 vol % of MeOH in DCM/MeOH, 70−100 vol % of ethyl acetate in heptane/ethyl acetate, 65−100 vol % of methyl acetate in heptane/methyl acetate, 50−100 vol % of acetone in heptane/acetone, and 70−100 vol % of dimethyl adipate in heptane/dimethyl adipate. To cover the composition range for achieving a R_f value of 0.6, three compositions of each of the first four solvent blends were selected (Table 2). The heptane/dimethyl adipate blend was not further pursued, as we observed very slow elution on the TLC plates due to dimethyl adipate's high viscosity, which is expected to translate into slow elution in actual column chromatography experiments.

For each of the solvent blends listed in Table 2, a solution containing mixtures of all four analytes dissolved in acetone, each at 3 mg mL^{-1}, along with three other solutions solely containing one of ibuprofen, aspirin, and acetaminophen in

acetone at the same concentration of 3 mg mL^{-1}, was then parallelly spotted on a TLC plate. It was found that in each experiment, the R_f value of each compound spotted from the mixture solutions was very close to the one spotted from the individual solution. The R_f values of the four analytes spotted from the mixture solutions eluted with three compositions of DCM/MeOH listed in Table 2 are compared to those eluted with the heptane/safer solvent blends, as shown in Figure S3. The R_f values in this figure are agreeable with those shown in Figure 2 at the same solvent compositions. Note that although we did not attempt to repeat our TLC experiments under precisely the same conditions, these agreements in R_f values demonstrate reproductivity of the experiments.

To select the solvent blends that would be used for subsequent column chromatography experiments, the R_f values of ibuprofen, acetaminophen, and caffeine shown in Figure S3 were extracted and replotted in Figure 3. Since for each API, three blend ratios of each heptane/safer solvent and DCM/MeOH were examined (Table 2), a total of nine R_f values were plotted for each API in each subfigure while comparing DCM/
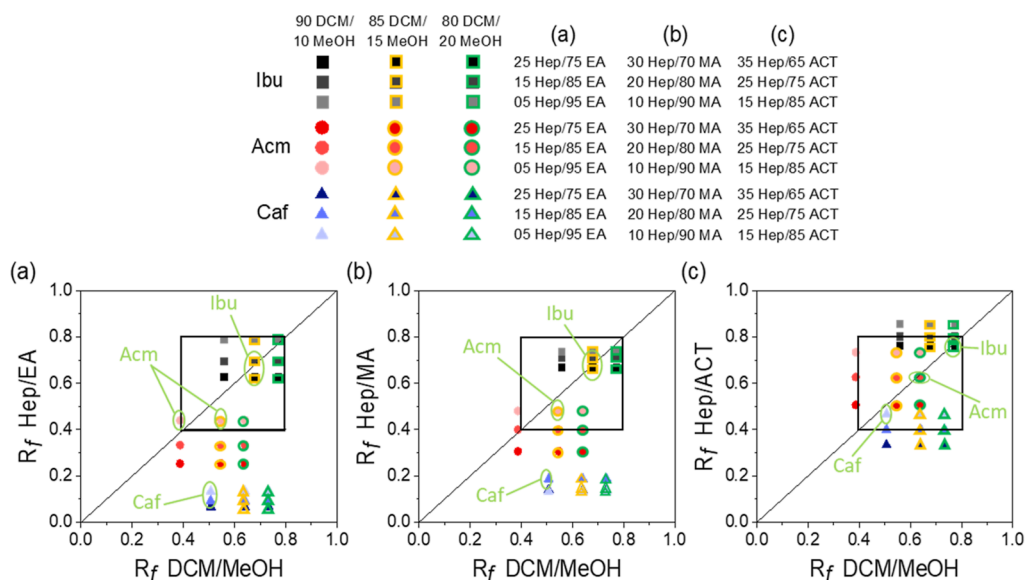
Figure 3. Determination of the solvent blend compositions for column chromatography tests by comparing R_f values of APIs obtained from (a) DCM/MeOH versus heptane/ethyl acetate (Hep/EA), (b) DCM/MeOH versus heptane/methyl acetate (Hep/MA), and (c) DCM/MeOH versus heptane/acetone (Hep/ACT).

Table 3. Blend Ratios for DCM/MeOH and Heptane/Safer Solvents That Were Further Tested in Actual Column Chromatography Experiments[a]

solvent blend	blending ratios tested (vol %)	cost ($/L)	Hansen solubility parameters (MPa$^{1/2}$)			dielectric constant
			δD	δP	δH	
DCM/MeOH	87.5% DCM/12.5% MeOH	107.7	16.7	7.9	9.0	11.94
heptane/ethyl acetate	10% heptane/90% ethyl acetate	134.8	15.8	4.8	6.5	5.66
heptane/methyl acetate	20% heptane/80% methyl acetate	118.8	15.5	5.8	6.1	6.04
heptane/acetone	25% heptane/75% acetone	145.0	15.5	7.8	5.3	16.24

[a]Cost, HSPs, and dielectric constants are calculated by taking the volume-weighted average of the values in Table 1.
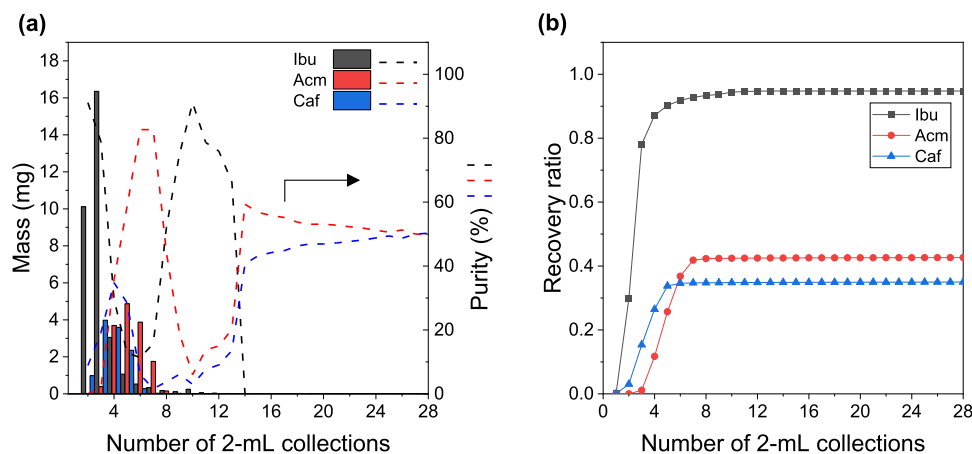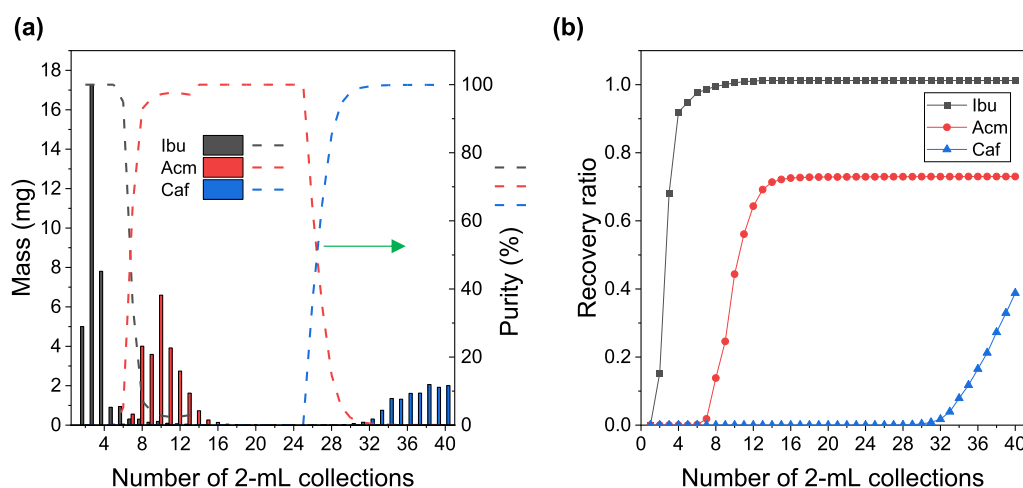

Figure 4. Separation of ibuprofen (Ibu), acetaminophen (Acm), and caffeine (Caf) with 87.5 vol % DCM in MeOH: (a) eluted mass (bars) and purity (dashed lines) and (b) accumulated recovery ratios for the three compounds, all plotted as functions of 2 mL collections. The column was operated at a 1:50 compound-to-silica gel ratio.

MeOH against each of the three heptane/safer solvent blend formulations.

The optimal solvent blends selected should have a R_f value within 0.4−0.8 and closest to the diagonal line, which would indicate similar performance between DCM/MeOH and heptane/safer solvent using column chromatography. To represent the best range of $0.4 \leq R_f \leq 0.8$, a box is shown in each of the subfigures in Figure 3. When several points were

found to meet the criteria (i.e., within the boxes) for a given API, as is the case with ibuprofen in Figure 3a, all of the points were retained. When no R_f values were found within the boxes for an API, as is the case with caffeine in Figure 3a−b, the point closest to the diagonal line was retained. After examining all of the suitable points for acetaminophen, ibuprofen, and caffeine in Figure 3, the mean values for all of the retained points were calculated and adopted for each DCM/MeOH−

Figure 5. Separation of ibuprofen (Ibu), acetaminophen (Acm), and caffeine (Caf) with 10 vol % heptane in ethyl acetate: (a) eluted mass (bars) and purity (dashed lines) and (b) accumulated recovery ratios for the three compounds, all plotted as functions of 2 mL collections. The column was operated at a 1:50 compound-to-silica gel ratio.

Table 4. Euclidean Distance (R_a) between Each of the Three Chemical Compounds (Ibu, Acm, or Caf) and Silica, the Column Stationary Phase, or One of the Six Solvent Blends Studied in This Work, Consisting of DCM, MeOH, Heptane (Hep), Ethyl Acetate (EA), Methyl Acetate (MA), or Acetone (ACT)[a]

chemical compound	R_a(MPa$^{1/2}$)						
	silica	87.5% DCM in MeOH	10% Hep in EA	100% EA	20% Hep in MA	100% MA	25% Hep in ACT
ibuprofen (Ibu)	13.0 (86.0)	5.9 (92.1)	4.4 (52.1)	4.6 (61.2)	5.5 (59.5)	6.3 (74.6)	7.1 (74.2)
acetaminophen (Acm)	3.9 (82.4)	6.0 (43.6)	10.2 (56.1)	9.4 (55.5)	10.2 (46.1)	8.5 (39.0)	10.1 (26.7)
caffeine (Caf)	6.7 (53.5)	7.2 (30.5)	11.2 (47.4)	10.6 (45.5)	11.4 (37.7)	10.1 (28.8)	11.3 (20.3)

[a]Values in the parentheses represent the percent contribution of R_a from the polarity (δP) term.

heptane/safer solvent pair as the final blending ratios to be further used in actual column chromatography experiments. Following this procedure, it was determined that 87.5 vol % DCM in MeOH, 10 vol % heptane in ethyl acetate, 20 vol % heptane in methyl acetate, and 25 vol % heptane in acetone are the most robust compositions of the solvent blends for the APIs studied. These four solvent blends (listed in Table 3), along with pure ethyl acetate and methyl acetate, were thus selected for actual column chromatography experiments, as discussed in the next section.

3.2. Solvent Performance Evaluated Using Actual Column Chromatography

In the present study, two metrics were selected to evaluate the performance of a heptane/safer solvent blend against that of DCM/MeOH in its ability of API separation and purification using column chromatography: (1) recovery ratio, which is the fraction of the compound recovered through the process, and (2) purity of the APIs recovered. It is envisioned that the performance of the solvents or solvent blends would be affected by the affinity of the compounds to the column stationary phase relative to that of the solvent (i.e., the column mobile phase). As a result, our column chromatography experiments were designed to test the hypothesis that the performance metrics are dependent on the HSPs of the chemical compounds, the mobile phase, and the stationary phase as well as the polarity (described by dielectric constants) of the solvents or solvent blends.

Figure 4 illustrates representative experimental results from column chromatography for the separation of a mixture of ibuprofen, acetaminophen, and caffeine. When the benchmark 87.5 vol % DCM/MeOH blend was used as the mobile phase,

ibuprofen was eluted first, followed by caffeine and acetaminophen. No eluted 2 mL fractions depicted a purity of 100% for any of the compounds (Figure 4a) since all three compounds were eluted together in relatively short amount of time, suggesting unsatisfactory separation under this operation condition. It was also observed that about 95, 43, and 35% of the loaded ibuprofen, acetaminophen, and caffeine were eluted and recovered from the process, respectively (Figure 4b).

Figure 5 shows the separation of the same mixture using 10 vol % of heptane in ethyl acetate. A better separation of the three compounds was observed, where high purities (>94%) of the APIs were measured in most 2 mL collections (Figure 5a). The purification process achieves recovery ratios for ibuprofen, acetaminophen, and caffeine of 100, 73, and 38%, respectively, higher than those using DCM/MeOH. The results using other heptane/safer solvent blends tested, along with using pure ethyl acetate and methyl acetate, are shown in Figures S4–S7. Note that although we did not attempt to repeat our column chromatography experiments, smooth trends were obtained from consecutive 2 mL collections in each experiment, suggesting reproducibility. Overall, the safer solvents or solvent blends provided better separation of the mixtures compared to the DCM/MeOH blend, although larger volumes were typically needed to achieve such a separation performance.

The order of elution, separation, and recovery ratio of each chemical compound coincides with the Euclidean distance (R_a) values derived from the HSP values (Table 4), which describe the distance between the compound to silica (the column stationary phase) and that to the solvent (the mobile phase) in a three-dimensional HSP space.[31,46] For a given chemical compound, a larger R_a to the column stationary phase
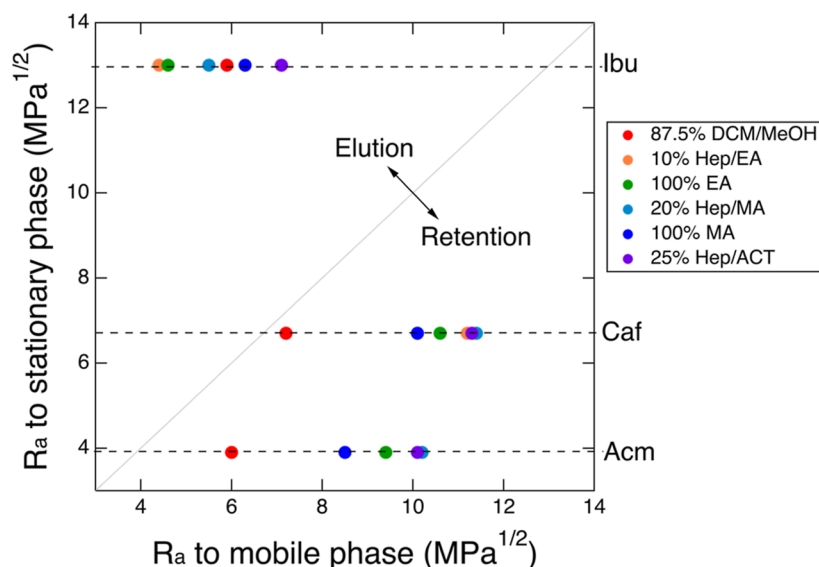
Figure 6. Parity plot comparing R_a values to the stationary and mobile phases for ibuprofen (Ibu), acetaminophen (Acm), and caffeine (Caf) eluted with different solvents or solvent blends.
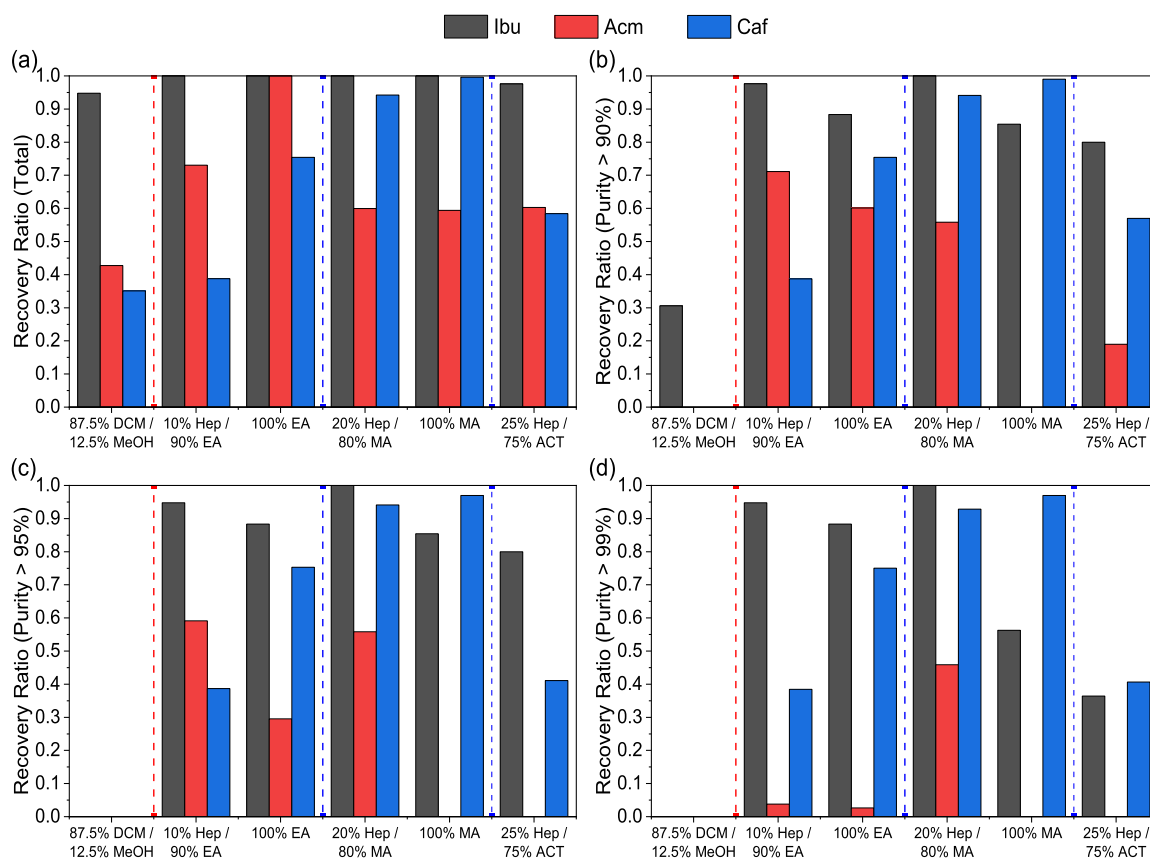

Figure 7. Comparison of recovery ratios of ibuprofen (Ibu), acetaminophen (Acm), and caffeine (Caf) in column chromatography when various solvents and blends were used as the mobile phase: (a) no purity requirement is imposed (total amount of compound recovered), (b) purity of >90% is imposed, (c) purity of >95% is imposed, and (d) purity of >99% is imposed.

compared with that to the mobile phase would favor its elution from the column. On the contrary, a larger R_a to the mobile phase compared with that to the stationary phase would favor its retention in the column. Comparing the R_a values across the compounds also gives an understanding of how the compounds would separate for a given stationary phase/mobile phase combination. Based on this principle, one can

generate a parity plot comparing the two R_a values for each compound/mobile phase combination, as shown in Figure 6, where points toward the upper left corner from the diagonal line represent the combinations favoring elution, while points toward the lower right corner represent those favoring retention. Our analysis shows that all of the points associated with ibuprofen are positioned near the upper left corner due to
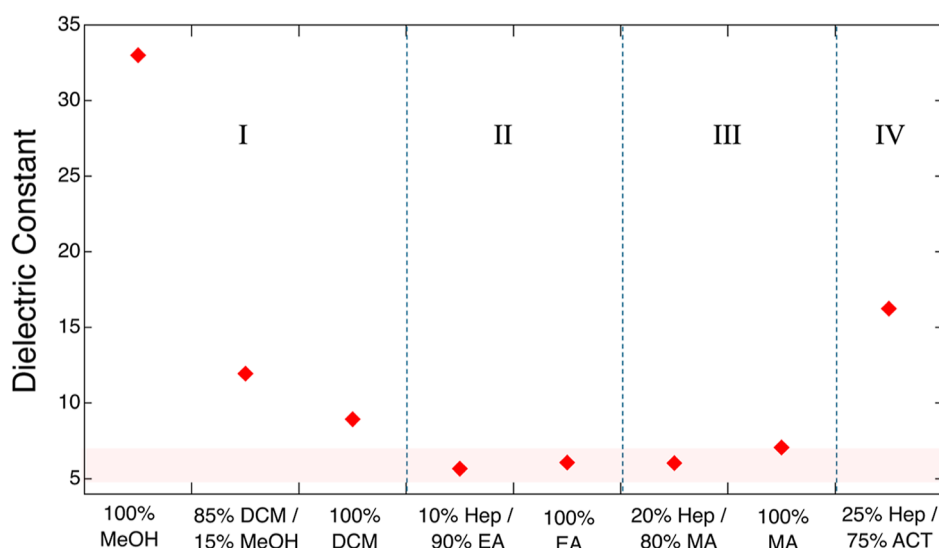
Figure 8. Optimal dielectric constant region (shadowed) for solvents to favor separation among ibuprofen, acetaminophen, and caffeine. Region I depicts the solvents to be replaced, including pure DCM, the 87.5 vol % DCM in MeOH blend tested in this work, and pure MeOH. Region II depicts safer solvents tested involving ethyl acetate (EA). Region III depicts safer solvents tested involving methyl acetate (MA). Region IV depicts the 25 vol % heptane/acetone (Hep/ACT) blend tested involving the very polar ACT.

its small R_a to the solvents tested (between 4.4 and 7.1 MPa$^{1/2}$) and a large R_a to silica at 13.0 MPa$^{1/2}$ (Table 4). This suggests that ibuprofen has a much higher affinity to the solvents compared to that to the silica gel stationary phase. It was thus not surprising in our column chromatography experiments that ibuprofen was always the first compound eluted with high recovery ratio regardless of the types of solvents used. Acetaminophen was typically the second compound eluted since its R_a values to the solvents are typically smaller compared to caffeine, despite its smaller R_a to the silica gel stationary phase (3.9 MPa$^{1/2}$, compared to caffeine at 6.7 MPa$^{1/2}$). This suggests that the effect caused by the mobile phase (hence, R_a to the mobile phase) could be more important. The DCM/MeOH blend was the only exception, where both compounds were eluted almost simultaneously with very similar recovery ratios. This suggests that the effect of R_a is nonlinear, where the same difference between two larger R_a values could cause more impact, as opposed to that between two smaller R_a values.

The separation of the three compounds for a given solvent or solvent blend could also be explained by the R_a values in Table 4 and Figure 6. For instance, when the DCM/MeOH blend was used, all three compounds have similar R_a values to the mobile phase, particularly between ibuprofen and acetaminophen (5.9 versus 6.0 MPa$^{1/2}$, respectively), causing them difficult to separate and resulting in low purity. The separation between ibuprofen and acetaminophen was improved when pure methyl acetate and 25 vol % heptane in acetone were used (where the differences in R_a values to the mobile phase were 2.2 and 3.0 MPa$^{1/2}$, respectively), although some overlap still existed. The separation was near perfect when 10% heptane in ethyl acetate, pure ethyl acetate, and 20% heptane in methyl acetate were used, where the differences in R_a values to the mobile phase were significantly larger at 5.8, 4.8, and 4.7 MPa$^{1/2}$, respectively. The separation between acetaminophen and caffeine was typically not an issue, except for DCM/MeOH where the R_a of acetaminophen and caffeine to DCM/MeOH are significantly smaller (at 6.0 and

7.2 MPa$^{1/2}$, respectively). This again suggests that the effect of R_a becomes more marked when the R_a values become larger.

The performance of the heptane/safer solvent blends in actual column chromatography against DCM/MeOH for the separation of ibuprofen, acetaminophen, and caffeine is summarized in Figure 7. The figure also illustrates that if recovered compounds are required to have higher than 90, 95, and 99% purity for each 2 mL collection, the amounts of APIs recovered successively drop. For instance, the 87.5 vol % DCM in MeOH blend was able to recover more than 95% of ibuprofen if no purity requirement was imposed (Figure 7a). However, when a purity requirement of >90% was imposed for each 2 mL collection from column chromatography, the 87.5 vol % DCM in MeOH blend could only recover 30% of ibuprofen since fewer 2 mL collections could meet this requirement (Figure 7b), and no 2 mL collections could meet higher ibuprofen purity requirements of >95 or >99% (Figure 7c−d). These results suggest that there exists a compromise between compound recovery and purity, highlighting the importance of good compound separation. Note that our column chromatography experiments also showed that larger amounts of solvents may be needed to achieve better separation by using safer solvents or solvent blends. This may increase the cost of solvent use and adversely reduce the benefits of employing safer solvents. As a result, one needs to carefully consider the trade-offs between the economic gains of higher API purity and the economic loss of larger amounts of solvents used, as well as the overall environmental and health impacts of using more solvents, before a decision is made.

To enhance the separation of the compounds, more silica gel can be added to increase the column height, which increases the retention time and enables more interaction between the APIs and stationary phase for separation. The performance of solvent blends that have demonstrated poorer separations in our work, e.g., 87.5 vol % DCM in MeOH and 25 vol % heptane in acetone, was further investigated by increasing the loading of silica gel into the column from 5 to 15 g. With increased amount of silica gel (taller column), both solvent blends show improved recovery ratios given the same imposed

purity requirements (Figure S8). The separation of the compounds was also improved (Figures S9 and S10) compared to the columns with less silica gel (Figures 4 and S7) due to the increased retention time and thus interactions between the APIs and the silica gel. Our results show that the amounts of silica gel (i.e., stationary phase) used could be an operation parameter to tune the performance of the solvents for API purification using column chromatography.

Since polarity is the major contributor to the HSP values of the compounds, mobile phases, and stationary phase of our system (the percentage in the parentheses in Table 4), one may simply use the polarity of the solvents or solvent blends, expressed by their dielectric constants, to predict if a solvent or solvent blend would provide satisfactory performance. Figure 8 shows the dielectric constants for the different solvents and solvent blends used as the mobile phase in the column chromatography experiments in this work. We noticed that when the solvents or blends have dielectric constant values between 5.66 and 6.08 (i.e., 10 vol % heptane in ethyl acetate, pure ethyl acetate, and 20 vol % heptane in methyl acetate; see Tables 1 and 3), the best separation of the compounds was observed (i.e., compounds were separated with high purities; see Figure 7). This may suggest that solvents or solvent blends within this narrow range possess the ideal properties (e.g., Euclidean distances between the APIs and the solvents) to achieve good separation and purification performances of the compounds studied. Future investigations are needed to further test this hypothesis by obtaining quantitative correlations between the HSP values of the APIs, solvent dielectric constants, and Euclidean distances between the APIs and the solvents.

4. CONCLUSIONS

In this work, several safer solvents and heptane/safer solvent blends were considered alternatives to DCM/MeOH for API purification by using column chromatography. Four polar safer solvents of interest, ethyl acetate, methyl acetate, acetone, and dimethyl adipate, were selected and blended with nonpolar heptane to tune the blend polarity. The DCM/MeOH and heptane/safer solvent blends were first screened using TLC to determine the blend compositions that satisfied the industrially recommended R_f values for optimal compound elution. The determined solvent formulations were subsequently tested in actual column chromatography using ibuprofen, acetaminophen, and caffeine as model compounds for their performance in API separation and purification. In general, the examined heptane/safer solvent blends provided higher API recovery and purity compared to those of DCM/MeOH in addition to potential health, safety, and environmental benefits. Of the heptane/safer solvent blends tested, 10 vol % heptane in ethyl acetate and 20 vol % heptane in methyl acetate demonstrated the best performance to replace DCM/MeOH. The Euclidean distance of each compound to the mobile phase and the polarity of the mobile phase were found to be critical parameters determining solvent performance. Our work demonstrates that there are possible inexpensive and safer solvents as promising replacements for DCM/MeOH, thereby potentially addressing a critical safety concern in API production in the pharmaceutical industry.

ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsenvironau.4c00015.

Sample HPLC chromatograms; HPLC calibration curves; R_f values from selected TLC experiments; and figures showing separation of APIs using different solvents or blends at 1:50 and 1:150 compound to silica gel ratios (PDF)

AUTHOR INFORMATION

Corresponding Author

Hsi-Wu Wong − *Department of Chemical Engineering, University of Massachusetts Lowell, Lowell, Massachusetts 01854, United States;* ⓘ orcid.org/0000-0003-2623-6145; Phone: +1-978-934-5290; Email: hsiwu_wong@uml.edu

Authors

Christian Ayafor − *Energy Engineering Program, University of Massachusetts Lowell, Lowell, Massachusetts 01854, United States;* ⓘ orcid.org/0000-0002-2831-4891
Toren Burton − *Department of Chemical Engineering, University of Massachusetts Lowell, Lowell, Massachusetts 01854, United States*
Nathaniel George − *Department of Chemical Engineering, University of Massachusetts Lowell, Lowell, Massachusetts 01854, United States*
Gregory Morose − *Massachusetts Toxics Use Reduction Institute, Lowell, Massachusetts 01854, United States*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acsenvironau.4c00015

Author Contributions

CRediT: **Christian Ayafor** data curation, formal analysis, investigation, methodology, validation, writing-original draft; **Toren Burton** data curation, formal analysis; **Nathaniel George** data curation, formal analysis; **Gregory Morose** conceptualization, methodology, project administration, supervision, writing-review & editing; **Hsi-Wu Wong** conceptualization, funding acquisition, methodology, supervision, writing-review & editing.

Notes

The authors declare no competing financial interest.

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