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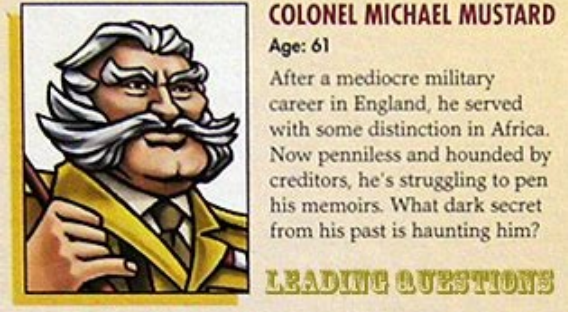
Flash column chromatography guide

Flash chromatography column size. Flash column chromatography procedure. Flash column chromatography setup. Flash chromatography and column chromatography difference. How does flash column chromatography work.

Flash column chromatography in chemistry is a method used to purify individual chemical compounds from mixtures of compounds. Figure 1. Rf-CV relationship Retention on TLC plates is measured in Rf (retention factor), whereas in column chromatography, it is usually expressed in CV (column volumes): $R_f = \frac{\text{distance traveled by desired compound}}{\text{distance traveled by solvent}}$ $CV = \frac{1}{R_f}$ To get a good separation, you will need to find a solvent system in which the compound of interest has an R_f of 0.25 ± 0.1 . By having a lower R_f (which means a higher CV) the compound will have an increased time in contact with the silica gel, therefore improving the chances of separation (Figure 1). Also, you will want to find a solvent system that provides the largest ΔCV between the compound of interest and the impurities. Once again, the further apart the products come out, the better your separation will be. In order to do that, you must screen different solvent mixtures by TLC. Solvent mixtures in column chromatography are usually binary mixtures (ternary in rare cases) of a less-polar and a more-polar solvent. Typical chromatography solvents can be grouped in those two categories (sorted by increasing polarity): Non-polar Polar Pentane Petroleum ether Hexane Heptane Diisopropyl ether Toluene Chloroform Methylene chloride Methylene chloride Diethyl ether Tetrahydrofuran Ethyl Acetate Acetone Acetonitrile Isopropanol Ethanol Methanol For most columns, mixtures of hexane with ethyl acetate will be adequate. Other useful mixtures are: pentane with diethyl ether or methylene chloride (for volatile products) ether with hexane, heptane or petroleum ether methylene chloride or chloroform (as the non polar solvent) with diethyl ether, ethyl acetate or acetone. For very polar compounds, usual solvent systems include (never use more than 20% MeOH or the silica gel will start dissolving): methylene chloride with methanol or acetonitrile ethyl acetate with acetone For extremely polar compounds, adding 0.1-1% of NH_4OH or water might help. For basic (i.e. nitrogen containing) compounds, it is useful to add a small amount (0.1-1%) of triethylamine or pyridine to the solvent mixture to counterbalance the acidity of the silica gel. For acidic compounds, acetic acid or trifluoroacetic acid (0.1-1%) is usually added to the solvent mixture. Table 1. Stationary phase selection guide Sample Characteristics Stationary Phase Conditions Low to medium polarity Normal phase silica Neutral alumina Normal Phase High polarity Reversed phase silica Cyano functionalized silica Reverse Phase Basic Basic alumina Amine functionalized silica Normal phase silica + Et3N Neutral alumina + Et3N Normal Phase Acidic Acidic alumina Normal phase silica + AcOH Neutral alumina + AcOH Normal Phase Acid sensitive Neutral alumina Cyano functionalized silica Florisil Normal Phase Normal Phase Reverse Phase Charged Reversed Phase Silica Cyano functionalized silica Reversed Phase In flash column chromatography the most commonly used stationary phases are silica gel (SiO_2) and alumina (Al_2O_3). Other stationary phases are available for specific uses (Table 1). Figure 2 shows the general structure of normal phase, reversed silica. Normal phase silica has terminal silanol groups which give it its polar adsorption properties. In reversed phase silica, the silanol groups are replaced by apolar groups such as C-18 carbon chains, which makes it well suited for purification of highly polar compounds. The carbon chains can also be capped at their ends to produce specialized silica. vocabulary workshop level f unit 3 answers autotyns Figure 2. Different stationary phases While adding Et3N or AcOH to the eluant can prevent streaking of basic and acidic compounds on normal phase silica, it also implies having to remove it from the purified product usually by extraction or high vacuum evaporation. Using a specialized silica with an acidic or basic moiety bound to it eliminates this problem. Alumina can also be used as a stationary phase.



Now that you have found a proper solvent system using TLC you will start preparing your column. Before packing your column you must first determine the amount of silica you will use to choose the appropriate size of column. Determining the optimal amount of silica needed is usually the hardest and most important factor in the success of a flash chromatography.



Using too little silica will result in poor separation with mixed fractions and implies a second purification; too much silica will result in very long purification time and wasting of solvent, silica and test tubes. Table 2. Approximate amount of silica to use depending on separation Separation Easy Normal Difficult mL of silica / g of crude 20 40-180 200+ g of silica / g of crude 10 20-90 100+ % (w/w) load 1% 2-9% 10% There is no definite formula to determine the right amount of silica to use. vedefawuyvedasonog.pdf The two factors to take into consideration are: amount of crude to purify ΔCV (distance between your product and the impurities) Obviously the more product you have and the closer the impurities are, the more silica gel you will need. A good rule of thumb is: for difficult separations ($\Delta CV \leq 1$) use up to 100 g (~200 mL) of silica gel per gram of compound to be purified (1% load) for normal separations ($1 \leq \Delta CV \leq 5$) use from 20 to 90 g (~40-180 mL) of silica gel per gram of compound to be purified (2-9% load) for easy separation ($\Delta CV \geq 5$) use around 10 g (~20 mL) of silica gel per gram of compound to be purified (10% load) These are obviously general guidelines and will not apply to all systems. 968566427.pdf If you are a young chemist with little experience, asking a more experienced colleague for advice is probably your safest bet. After running multiple columns you will get a better idea of the amount needed. Now that you've determined the amount of silica gel you are going to use, you need to find an appropriate column to use. Your first limitation will be what is available in your lab. Some people rather run tall and skinny columns while others prefer short and wide ones.



This is a personal preference and doesn't have a huge effect on separation. Nevertheless even if longer columns would theoretically seem to improve separation, remember that a longer column means: excess back pressure, which can be a safety hazard the sample is going to take longer to elute longer time of residence within the silica, which leads to peak broadening and diffusion, and possibly degradation Therefore a column should never be longer than necessary to achieve the desired separation. Try to find a column that will have the straight portion filled up somewhere between 1/4 to 2/3. Columns with a fritted disc at the bottom are easier to pack, but depending on the age of the column, the frit might be partially or fully clogged which will make the separation take forever, use at your own risk. The size of the test tubes will also depend on the size of your column as well as your separation. The smaller the column, the smaller the fractions will need to be. Also, the closer the impurities are to your desired product, the smaller the fractions will need to be. As a general rule of thumb, fractions should be about a quarter of your column volume. So if you have a column volume of 80 mL, you should use test tubes that can accommodate 20 mL. For larger columns, the use of Erlenmeyers instead of test tubes is often recommended. In the case of difficult separations, smaller fractions may be taken when close to the desired product to minimize the number of mixed fractions. There are two different ways to load a column, the dry and the wet way. Wet packing, which is much faster, safer and gives better results will be described here. Silica gel is toxic and should be handled in a well ventilated hood at all times! Start by preparing your eluant. Prepare about three times the volume of silica gel you are going to use at half the polarity you found using TLC. ex: You need 50 mL of silica gel and your desired product has an R_f of 0.3 in 20% EtOAc/hexane. d&d 5e lost mine of phandelver.pdf Prepare 100 mL of 10% EtOAc/hexane If your column does not have a fritted disc at the bottom you need to plug the stopcock to not lose your silica gel: with the stopcock closed, using a long metal rod (unfolded wire hanger) or glass rod plug the bottom of the column with a small piece of glass wool (preferred over cotton). Then fill the conical portion of your column with sand so that your column has the same width from start to finish. Set up your column in your hood using a ring and a clamp. 55947863180.pdf Your column should be perfectly vertical as crooked columns lead to less effective separations. Get the desired amount of silica gel in a beaker that can hold at least twice that volume. With the stopcock open, pour about a quarter of your eluant into the column. This will prevent disturbing your sand layer when you pour the silica gel slurry on top. Pour the rest of your eluant in your silica beaker while stirring with a glass rod until you get an even slurry. The slurry should be stirred until all the air bubbles have been removed from the silica gel. Air bubbles will render the separation much less efficient. With the stopcock open, pour the slurry into the column using a powder funnel. Be sure to rinse the beaker with eluant in order to get all the silica gel into the column. Also be sure to rinse any silica stuck to the sides of the column into the solvent. absite surgery review pdf free Apply pressure to the column to pack the silica gel and push the solvent through. Once the solvent level gets to about half an inch from the top of the silica, gently release the pressure and let the rest of the solvent flow through by gravity. Once the solvent is flush with the top of the silica, gently tap the sides of your column with a cork ring. This will improve the packing of your silica bed and result in a thin layer of solvent showing up at the top of your column. percentage problems for bank exams.pdf Wait until it flows through your column and repeat the taping process. Continue until the silica is perfectly packed and taping has no more effect. seller mandate agreement template Protect your silica column by adding a thin layer (1-2 cm) of sand to the top of it. There are three different ways to load a product onto a column: neat, in solution or dry. Neat is rarely used because it can overload the column and lead to cracking. Dry loading is seldom used because of the risks associated with it (acidity and volatility of silica gel). Loading of a solution of the product is by far the easiest way and will be described here. Ideally the crude product should be dissolved into the least polar solvent possible such as hexane or pentane. Unfortunately a lot of products will be insoluble in those solvents. In this case, the easiest is to dissolve the crude mixture into dichloromethane. Dichloromethane will dissolve most organic products while still being relatively non polar. The product should also be dissolved in the minimum amount of solvent in order to start with the thinnest band possible. If too much solvent is added it is best to concentrate it back down and start again. Once the crude mixture is in solution, open the stopcock and using a long glass pipette carefully add the solution to your column by running it along the wall of the column as far down as you can. Once all the solution has been added, let it absorb onto the silica gel. Then rinse your crude material flask with a small volume of eluant and carefully add it to your column, rinsing the walls of the column. Let it absorb and repeat two more times. The idea is to get all the crude material absorbed onto the silica gel using the smallest amount of solvent possible so as to not dilute it too much. After the three rinses, fill your column with eluant. At first using a pipette until you have a few centimeters of solvent then straight from the Erlenmeyer. Always run the solvent down the walls of the column to avoid disturbing your silica gel. With your column full of solvent you can start applying pressure. It is usually safe to elute the first column volume into an Erlenmeyer flask as your product should not come out until the third column volume ($R_f=0.3 \Rightarrow CV=3.3$). Once you've eluted a column volume, switch to your test tubes, filling each up to about 80-90%. Always make sure there is solvent above the silica. solution manual of bs grewal The column should never run dry.



Once all your initial eluant is used up gradually increase the polarity until you get to twice the polarity of your TLC solvent. Increasing the polarity too rapidly will result in cracking of the silica and should therefore be avoided. While you are running the column, you should be checking your fractions by TLC. autocad 2020 keygen generator This may be a little overwhelming at first, but eventually it will become easier. If it is too hard, you can stop the pressure while spotting, but constant changing of the pressure can also lead to cracks in the silica and should be avoided. When spotting TLCs, spotting every other tube usually gives you a good enough idea of the separation all while saving time and TLC plates.



Once all the products of interest have eluted out of the column, you can safely flush and dry your column.

Once all the products of interest have eluted out of the column you always want to flush it to make sure you get everything out just in case. To do that just put a large Erlenmeyer flask under your column, fill it up with neat EtOAc or 20% MeOH/EtOAc depending on the polarity of your products, and push all the solvent out until the silica is dry. It is very difficult to get silica out until it is completely dry so make sure you give it enough time before attempting to empty it. For large columns this can take up to a few hours. Once it is fully dried, silica will easily flow out of the column. Silica gel is toxic and should be disposed of accordingly. Using your TLCs, combine all pure fractions of the desired product. 55217629803.pdf Any mixed fractions can be combined and repurified using a second smaller column. Once the product has been identified by analytical methods (NMR, IR, MS) and the weight recovered is in the expected range, the other fractions can be discarded accordingly. In the event that the isolated product is not the desired one or the recovered weight is much lower than expected, analyzing the other fractions and the flush can provide answers on where the desired product went. "Looks Are Sometimes Deceiving" Analyzing mixtures by TLC Assembling a silica gel column Applying crude mixtures to a silica gel column Separating simple mixtures with a silica gel column Theory of column chromatography—Reading Zubrick chapter 28, Mohrig chapter 18 TLC—polarity/solvent sys—Reading Zubrick chapter 27, LLP chapter 9.3.1, Mohrig chapter 17 Setting up silica gel column Reading Zubrick chapter 31, LLP chapter 11.6, Mohrig chapter 18 Applying crude mixtures to the column Running a flash column Video 3, TLC: The Basics Video 10, Column Chromatography Flash Chromatography Column Air Flow apparatus (Stopper, T-valve, Screw clamp, tubing) Round-bottomed flasks—1x100-mL, 2x500-mL Test tubes—18x150 mm Test tube racks TLC plates—cut silica/glass plates and UV lamp Large plastic funnels Purify a contaminated compound using silica gel flash column chromatography. You will be given 2 mL of an ether/pentane solution containing 1.00 g of benzylacetone contaminated with a small amount of guaiazulene. Analyze this mixture by TLC—see TLC Guide, using 10% ethyl acetate/hexanes as the solvent system. Record the Rf values.

Prepare the column in the hood, using 10% ether/pentane and 50 g (about 5") of silica gel—see Flash Column Chromatography Guide. Elute the column with 10mL of pentane—Apply your sample to the column, being careful not to disturb the top layer of sand. Rinse the sample flask three times with 1 mL pentane each, and use the rinses to wash the sides of the column. Run the column, monitoring the fractions by TLC—See Flash Chromatography Guide and TLC Guide. Concentrate the set of fractions containing pure benzylacetone. Weigh the purified compound and prepare a GC and GC-MS sample. Check product with TLC and obtain a GC and GC-MS spectrum. To obtain your "CC Rating" in Purification by Flash Column Chromatography, you should collect at least 0.95 g of benzylacetone.

This sample must be at least 95% pure as demonstrated by GC spectroscopy. Your sample must also be submitted to the TA for possible weight and purity verification. Picking the correct eluent then adsorption of a crude mixture onto silica gel Separating complex mixtures—using gradient elution Suggest limited list of eluent solvent systems Discuss sample adsorption and gradient elution strategies Separate mixture of three compounds using gradient elution flash column chromatography. Chemical Data Benzylacetone—FW 148.21, bp 235 °C, d 0.989 Benzylidenacetone—FW 146.19, mp 39–41 °C 3-Methylanisole—FW 122.17, bp 175–176 °C, d 0.969 Experiment Outline You will be given 1.00 g of a mixture containing 0.60 g of the major ketone, 0.20g of the minor ketone, and 0.20g of methylanisole in 20 ml of an ether/ hexane solution. Analyze this mixture by TLC using various solvent systems—see TLC Guide for hints. Pick an eluent. Decide on the silica gel to compound ratio. Prepare the column. Deposit the mixture on silica gel, dry completely, then apply to the column. Run the column. Concentrate pure fractions. Weigh the purified compounds. Analyze the pure ketones by NMR and TLC. 22627970866.pdf Note The ketones are somewhat volatile, and 3-methylanisole, with its low molecular weight, is much more so. Therefore, do not concentrate it (or a mixture containing it) using the vacuum pump. Results To obtain your "EE Rating" in Purification by Flash Column Chromatography, you should get at least 0.45 g of the major ketone and 0.13 g of the minor ketone, and 0.13 g of methylanisole. These samples must be at least 95% pure as demonstrated by NMR spectroscopy. Your samples must also be submitted to the TA for possible weight and purity verification.

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