Reverse Phase Flash Chromatography: A Convenient Method for the Large Scale Separation of Polar Compounds

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Abstract: The application of Reverse Phase Flash Chromatography to the separation of mixtures of polar compounds such as amino acids, nucleosides, carboxylic and sulphonic acids is described.

Most synthetic transformations produce more than one product necessitating purification of the crude reaction mixture to obtain pure material. One of the most commonly used techniques for the purification of organic compounds is Flash Chromatography as formalized by Still[1]. Increasingly Organic Chemists are dealing with highly polar compounds and the purification of such materials is difficult using this technique, the only avaliable alternative being the use of reverse phase HPLC which is both very costly and time consuming. Herein we describe a method for the large scale separation of such polar compounds based on the use of dry flash chromatography as described by Harwood[2]. The use of reverse phase flash silica which can be prepared in a two step procedure or purchased directly[3] in conjunction with the dry flash technique which relies on suction rather than positive pressure allows the rapid purification of a variety of polar compounds.

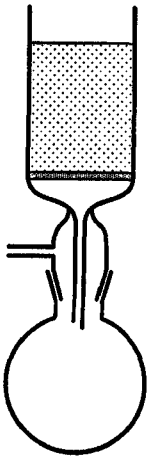
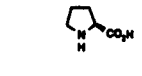
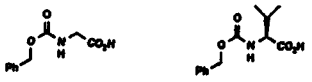


Figure 1

The apparatus used is shown in figure 1 above. A 5cm diameter, porosity 3 sintered funnel is used. The sintered funnel is filled to within 2 cms of the top with reverse phase silica (ca. 40g) The mixture of compounds to be purified is adsorbed onto a small amount (ca. 4g reverse phase silica/1g of mixture to be separated) by dissolving in a polar solvent (such as methanol) adding the reverse phase silica and removing the solvent using a rotary evaporator.

The reverse phase silica in the sintered funnel is levelled by gently tapping the sides of the funnel and then placed under vacuum. While a vacuum is maintained the mixture of compounds and reverse phase silica is added to the top of the column, the vacuum is released and the side of the funnel is again gently tapped to ensure the mixture just added is spread evenly over the surface. The vacuum is reapplied and the column is now ready for use. The vacuum is maintained while 20ml of the solvent mixture being used is added to the surface of the column. The solvent is sucked through and collected in the round bottomed flask. The vacuum is released and the fraction collected in the flask removed, the flask washed out with a small amount of the solvent mixture and the apparatus reassembled. The vacuum is applied before the next portion of solvent is added to the top of the column and the process repeated.

Table. Separation of Polar Compounds by Reverse Phase Flash Chromatography

Entry	Mixture	Eluant
1.		50% MeOH/50% H_2O
2.		40% MeOH/60% H_2O
3.		50% MeOH/50% H_2O
4.		10% MeOH/90% H_2O
5.		40% MeOH/60% H_2O
6.		30% MeOH/70% H_2O
7.		5% MeOH /95% H_2O
8.		H_2O
9.		80% MeOH/20% H_2O
10.		20% MeOH/80% H_2O

Examination of the table shown below shows a number of examples of separations carried out using this technique. In all the examples shown 1g of a 1:1 mixture of the two components was used.

The simplest type of separation involves that between a polar and a relatively non-polar compound, the polar compound eluting from the column first (for example proline and Z-proline, proline elutes first). Several other amino-acid derivatives are illustrated in examples 1-5. In entry 2, the purification of (Z)-glycine and (Z)-valine (Z = N-benzyloxycarbonyl) illustrates how separation can be achieved on the basis of a change in hydrophobicity of the compounds. Other amino acid derived compounds in examples 3-5 illustrate how highly polar compounds which differ in only one or two functional groups can be separated efficiently. Entries 7 and 8 show how this technique can be applied to the separation of nucleosides (A small amount of a mixed fraction was obtained in example 8). We are currently investigating the separation of other carbohydrate derived compounds using this method.

In entry 9 the betaine shown was readily separated from two unidentified components after repeated attempts to purify it using standard flash chromatography had failed[4]. Finally, entry 10 illustrates the separation of a carboxylic and sulphonic acid. It is also worth noting that triphenylphosphine oxide can be eluted (70% MeOH/30% H_2O) on a reverse phase flash column and this technique may offer an alternative procedure for the removal of this unwanted (usually) compound. This technique may also find use in the large scale purification of acid sensitive materials.

Preparation of Reverse Phase Silica.

Merck silica gel 60 (230-400 mesh) (500g) was placed in a two litre three necked round bottom flask equipped with an overhead mechanical stirrer, a nitrogen inlet and a reflux condenser with a gas outlet tube. The silica gel was suspended in dry toluene (1 litre) then octadecyltrichlorosilane (ODS) (50ml) was added and the resulting slurry was heated to reflux with stirring for 18 hours with dry nitrogen gently bubbling through the slurry. The mixture was allowed to cool to room temperature and the solvent decanted off. More dry toluene (500ml) was added, the contents of the flask shaken vigorously then allowed to settle. The toluene was decanted off and the process repeated with two more portions of toluene and then six portions of dry methanol (500ml each). The silica gel was filtered under suction and dried at 40° overnight.

The silica gel was resuspended in dry toluene (1 litre) in the apparatus described above and hexamethyldisilizane (50ml) was added. The suspension was heated to reflux, with stirring and a nitrogen purge as described above for two hours. The suspension was allowed to cool to room temperature and the washing procedure described previously was repeated. After filtration and drying at 40° overnight the reverse phase silica is ready for use.

Acknowledgment: I would like to thank the Nuffield Foundation and The James Black Foundation for generous financial support.

References and Notes

1. W.C.Still, *J.Org.Chem.*, 1978, *43*, 2923.
2. L.M.Harwood, *Aldrichimca Acta*, 1985,*18*, 25.
3. The Reverse-Phase Silica used in this procedure can be purchased directly from the Aldrich Chemical Company, Catalog No. 37763-5.
4. We would like to thank Prof. I.O.Sutherland for this sample.