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# Visualization of Column Chromatography

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***Abstract:*** A **method** is described which allows visualization of column chromatography by use of a quartz column and addition of a fluorescent indicator to commercial adsorbents.

During the past few decades, preparative organic chemistry has undergone a revolution owing to chromatographic techniques,' which have provided extremely versatile and efficient methods of separation and purification of organic compounds. Although advances such as preparative HPLC, automatic fraction collectors and radial chromatography have appeared on the market, perhaps the technique in greatest usage by the average synthetic chemist today is flash chromatography,2 due to its low cost and ease of operation. The ability to guage the elution of products would be a significant improvement of this technique. This has been addressed by radial chromatography.3 where a UV transparent cover allows visualization of compounds capable of quenching the fluorescence of an indicator present in the rotating stationary phase. Limitations of this system include the need for specialized equipment and gel, and a maximum loading of about **1.5g.**

The extension of this methodology to flash chromatography is possible by utilizing columns made of a grade of quartz which is largely transparent to UV radiation at 254nm. The stationary phase is prepared by simply stirring a small amount of fluorescent indicator" into a slurry of adsorbent (e.g. flash quality silica gel) before pouring it into the column. The elution of compounds can be readily followed by UV irradiation. Visualization occurs as purple bands on a green background. This result is due to compounds which quench the green fluorescence of the indicator allow the purple fluorescence of the quartz to become visible. The fluorescence of the quartz may blur the exact separation between two very close bands therefore taking several fractions at these points during elution may be necessary to monitor the separation. Although fluorescence-free quartz tubes are available, they are about ten times more expensive and they are thus probably not worth the slight increase in convenience.

The column consists simply of a quartz tube adapted on one end to fit a septum, so that pressure may be applied through a needle. Because quartz cannot be directly fused to **pyrex,** the stopcock is joined by connecting it with the narrowed end of the tube through a short piece of Teflon tubing. The total cost of a 12 mm (inside diameter) quartz column is twice that of a **commercial** Pyrex column while the corresponding 22mm columns is 4 times the price of the Pyrex equivalent.6 If a solvent reservoir is desired, it is also possible to purchase a quartz-to-Pyrex connector which can allow one to be fitted at the top of the column. Similarly, for larger columns a ground-glass adaptor can be mounted to insure that a sufficient solvent flow rate is maintained.

In a typical procedure, zinc silicate indicator[4] (7.5mg/g adsorbent) is added to a slurry of the adsorbent in the solvent system to be used and mixed until homogeneously fluorescent to a 254 nm UV lamp. Although some of the indicator may remain as small clumps this does not seem to appreciably affect performance. The remaining procedure is essentially that of conventional flash chromatography2 except that the eluting compounds can be observed as purple bands by irradiating the column with a UV lamp, preferably in a dark room. In many cases, less solvent is needed than in flash chromatography since solvent polarity can be quickly adjusted to yield the shortest elution time while still achieving separation of each band. In general, fractions need only be taken during the elution of each band, which will result in some reduction of TIC plates required and a quicker separation, compared to conventional flash chromatography.

Detection limits of less than 10 mg on a 12mm inside diameter column are typical, however this will depend on the activity of individual compounds. In general, the intensity of each band will correspond to how actively it quenches fluorescence on commercial indicating TIC plates. In a similar way, the choice of solvent systems can be decided by viewing a soaked TIC plate and ensuring that fluorescence is not quenched. Typical chromatography solvents such as hexanes, ethyl acetate, chloroform, methylene chloride, acetonitrile, ether and methanol are acceptable but acetone, benzene and toluene are not.

The quartz column offers significant advantages over currently used preparative chromatographic methods. First, this technique is applicable to the commonly used commercially available stationary phases. Thus far, both normal and reverse-phase silica as well as alumina have been used successfully. Second, the size of the load is limited only by the size of the quartz tube available. Third, the technique is very economical and requires no other special equipment, except a UV lamp. For these reasons the quartz column should prove to be both an economic and time-saving asset to the synthetic organic chemist.

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References:

(1) Hostettmann, K.; Hostettmann, M.; Marston, A. ***Preparative Chromatography Techniques.*** Springer-Verlag, New York, 1986.
(2) Still, WC.; Kahn, M.; Mitra, A. ***J.*** Org. ***Chem. 1978.43, 2923.***
***(3) see*** ref 1, p. 12.
(4) Activated zinc silicate (available from Sigma) is used, the same material found in commercially available TIC plates. See Barrett, G. C. in ***Advances in Chromatography (Ed.*** Giddings, J.C and Keller, R.A.), vol 11, Marcel Dekker, Inc., New York, 1974, p. 162.
(5) This column is capable of separating about **5g** of material based on the 20-30: 1 ratio (silica gel:compound) recommended in: Pasto, D. J.; Johnson, C. R. ***Laboratory text for organic chemistry,*** Prentice-Hall, New Jersey, 1979, p.63.
(6) TO8 commercial quartz tubes were obtained from Heraeus Amersil (650 Jernees Miln Rd., Sayreville, New Jersey, 08872).

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