

Lab Highlights

ISOCRATIC AND GRADIENT SEPARATION OF RED AND BLUE DYES FROM GRAPE KOOL-AID®¹ USING SEP-PAK® CARTRIDGES

Many of you have seen the photographs on our SEP-PAK^R cartridge literature showing a colorful separation of red and blue dyes on a SEP-PAK^R cartridge. Since we have received a number of calls requesting the conditions for that separation, it seems appropriate to present this information here².

Either a step gradient or an isocratic elution can be conveniently performed to separate the red and blue dyes in grape KOOL-AID^R, using inexpensive and relatively non-toxic solvents. The step gradient is most impressive from a visual standpoint, but the isocratic demonstration has the advantage that it can be used to calculate values for resolution, selectivity and even column efficiency. We usually measure resolutions of at least 1.0 and find approximate efficiencies of 50 plates or more.

A number of eluents have been used for this demonstration, but the use of isopropanol, water and household vinegar gives good results while limiting the toxicity of the solvents. Methanol and acetonitrile have been successfully used, although the exact concentrations will be different than for isopropanol. Five experiments are reported below, involving both silica and C₁₈ SEP-PAK^R cartridges. Of the two step gradients, the separation on C₁₈ gives sharper bands. The isocratic separations on silica SEP-PAK^R cartridges are particularly interesting, because the two dyes elute in the opposite order depending on whether water or vinegar is mixed with the isopropanol. When water is used, the elution order is the same as for the C₁₈ separations. By pretreating the cartridge with vinegar, on the other hand, it appears that the silica is modified, with a resulting change in the elution order. The nature of this modification may well involve the silanol sites.

We have found these demonstrations to be quite effective at illustrating the basic principles of liquid chromatography. Grape KOOL-AID is but one sample which can be used. Mouthwashes, after-shave lotions and various soft drinks might also be useful as samples, as long as two or more dyes are present.

Sample:	Unsweetened Grape KOOL-AID, 0.3 g/100 ml water
NOTE:	Solution must be fresh for best results
Injector:	1 ml Leur-LOK TM 3 disposable syringe
Pump:	10 ml Leur-LOK TM disposable syringe
Columns:	C ₁₈ and Silica SEP-PAK ^R Cartridges
NOTE:	To avoid "post-column band broadening": it may be helpful to cut off the exit tube of the cartridge.
Elution:	The red dye (FD&C #40) will elute before the blue dye (FD&C #1) for all demonstrations except 3. Less than 10 ml of eluting solvent will be sufficient for each separation.

CALCULATION OF k' , resolution, α and N :

By collecting the column effluent in a graduated cylinder, elution volumes and band widths can be measured. Once a value for the void volume is found, values for k' , resolution, α and plate count can be calculated by the usual equations.

APPROXIMATION OF THE VOID VOLUME (V_0):

For the calculation of k' values, an estimate of V_0 is needed. This can be approximated as 50% of the empty column volume. Since the column is cylindrical, measurement of the column length and diameter allows calculation of an approximate V_0 .

SEPARATION CONDITIONS FOR 5 DEMONSTRATIONS:

1. ISOCRATIC, C_{18} CARTRIDGE

Pretreat the cartridge by slowly pumping 10 ml of isopropanol followed by 10 ml of water. Inject 1 ml of sample. Elute both dyes by slowly pumping 16% IPA in water. The cartridge can be reused many times if the pretreatment is repeated.

2. ISOCRATIC, SILICA CARTRIDGE

Pretreat the cartridge with 10 ml of water. Inject 1 ml of sample and elute both dyes with 16% IPA in water. Repeat the pretreatment before any further injections.

3. ISOCRATIC, SILICA CARTRIDGE

Pretreat the cartridge with 50 ml of household distilled white vinegar. Inject 1 ml of sample and elute the dyes with 16% IPA in vinegar. (Note: if the blue dye does not elute first, continue to pretreat with vinegar until it does. This cartridge should not be used for Demos 2 or 5.) Between experiments, retreat the cartridge by passing 10 ml of vinegar through it.

4. STEP GRADIENT, C_{18} CARTRIDGE

Pretreat the cartridge as in Demo 1. Inject 1 ml of sample and elute the red dye with 5% IPA in water. Elute the blue dye with 25% IPA in water. Repeat the pretreatment between experiments.

5. STEP GRADIENT, SILICA CARTRIDGE

Pretreat the cartridge with 10 ml of water and inject 1 ml of sample. Elute the red dye with 100% water and the blue dye with 16% IPA in water. Treat with 10 ml of water between experiments.

1. KOOL-AID is a registered trademark of the General Foods Corporation.
2. B. A. Bidlingmeyer and F. V. Warren, Jr., J. Chem. Educ., in press.
3. Luer-LOK is a registered trademark of Becton-Dickinson & Co.