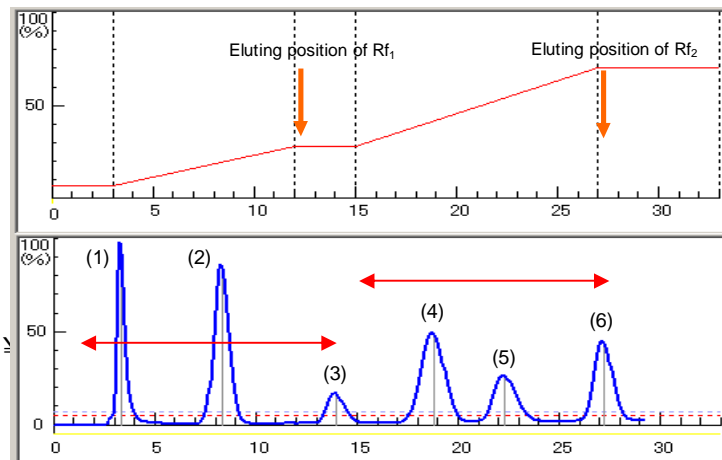


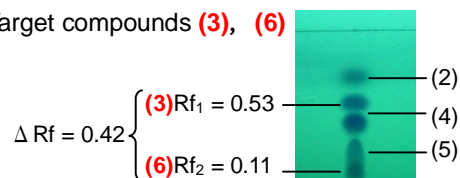
## AUTOMATED 2-STEP GRADIENT ELUTION METHOD IMPROVES PURIFICATION & SEPARATION OF MULTIPLE COMPOUNDS EFFICIENTLY.

2-Step Gradient is the best method to use when separating two target compounds whose TLC Rf values ( $R_{f1}$  &  $R_{f2}$ ) (3) & (6) are far apart from each other ( $\Delta R_f > 0.3$ ) with some other compounds in between. As Fig 1 shows, 2-Step Gradient purifies multiple compounds efficiently while achieving a high resolution. And  $R_{f1}$  (Sample 3) and  $R_{f2}$  (Sample 6) elute at 4 to 5 column volume on each gradient.

**<Fig 1>** Column: High-Flash, L (30g) Flow rate: 20ml/min.  
**Method: Automated 2-Step Gradient**  
 Target compounds:  $R_{f1} = 0.53$  (Sample 3),  
 $R_{f2} = 0.11$  (Sample 6) To (1-CV) = 3 min.



Target compounds (3), (6)



Solvents

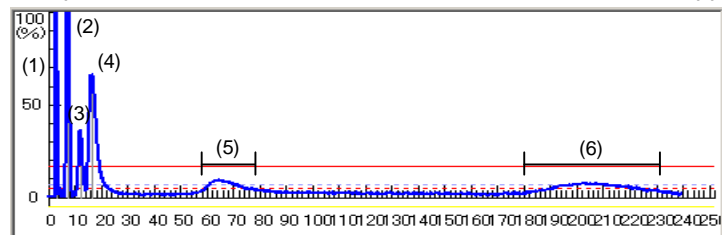
Hexane : Ethyl acetate = 7:3

- 1: Toluene (Non-absorption)
- 2: N-Methyl aniline ( $R_f = 0.71$ )
- 3: Dimethyl phthalate ( $R_f = 0.53$ )
- 4: Butyl-P-HO-benzoate ( $R_f = 0.42$ )
- 5: 2-Hydroxyethyl Salicylate ( $R_f = 0.26$ )
- 6: Nifedipine ( $R_f = 0.11$ )

Resolution and Shape of Peak: Excellent  
 Run time: 28 minutes. Short and efficient.  
 Solvent consumption: 560ml. Economical and **eco-friendly**.  
 Workload: **Input Rf values of two target compounds and information on solvents, choose the gradient mode, equilibrate the column, and then start run. It's that simple and easy to operate the system.**

**<Fig 2>**

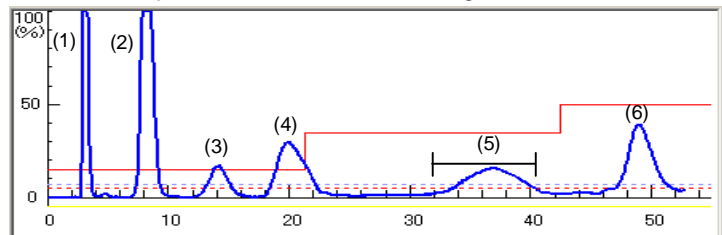
The conventional ISOCRATIC method takes a long time to elute high polar compounds with small Rf values. Sample 6 eluted at the same solvents mixture ratio that was applied to the separation of Samples 1, 2, 3, and 4.



Resolution: Fair  
 Peak shape: Broad  
 Run time: 240 minutes. A long run  
 Solvent consumption: 4800ml. A lot of solvents consumed.  
 Work load: Peaks for Samples 5 and 6 are so shallow and broad, therefore, they cannot be well observed.

**<Fig 3>**

Conventional Step-wise Elution method relies on experience. Automatic method set up and automated operations are impossible. Manual manipulation of the solvent strength and increase for each compound needs to be determined.



Resolution: Good  
 Peak shape: Fair  
 Run time: 52 minutes. Shorter than ISOCRATIC, but longer than 2-Step Gradient.  
 Solvent consumption: 1040ml. Almost two times as much as 2-Step Gradient.  
 Work load: Chemist cannot leave the site while running a sample, has to check eluting peaks and need to determine and increase the solvent strength for high polar solvents. No prediction of where the target compounds elute. This method would require a lot of experiences in determining the best-suited solvent mixture for each sample. Therefore, extra workload.

	ISOCRATIC	Step-wise	Automated 2-Step Gradient
Run time & Solvent Consumption	240min, 4800ml	52min, 1040ml	<b>28min, 560ml</b>
Work load	Heavy	Very heavy	<b>Light</b>
Experience in chromatography	Required	Required	<b>Not required</b>