The Choice of Mobile Phase in Reversed-Phase Flash Chromatography

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Abstract

Reversed-phase separation mode is the preferred method for the separation and purification of most polar compounds. Comparing with other separation modes, reversed-phase separation mode is more universal and convenient, thus satisfying results are usually obtained. In practice, it cannot be neglected that the type and polarity of the mobile phase have strong impact on the separation effect. In this application note, the impact on the separation effect for the same sample by using three different mobile phases was described in detail, as well as how the eluting gradient could influence the separation procedure.

Introduction
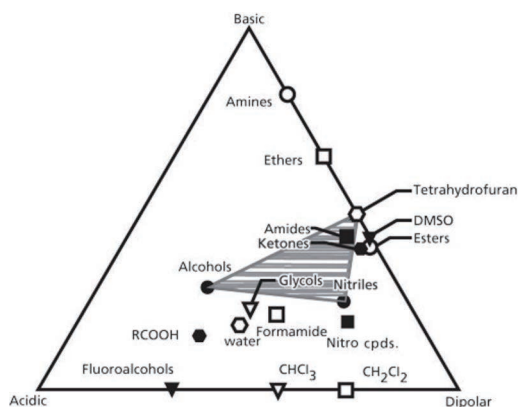
Figure 1. Classification of solvent properties according to their acidic, basic, and polar nature. Reproduced from reference 1.

There are many kinds of mobile phases could be used in reversed-phase separation mode (as shown in Figure 1). For example, water < methanol (MeOH) < acetonitrile (ACN) < ethanol (EtOH) < tetrahydrofuran (THF) < isopropanol (sorted by polarity). Due to the limitation of time and other factors, it is impossible for us to try all kinds of mobile phases in practical terms. Considering relevant factors such as selectivity, economics and safety, it is recommended to try the following solvents as the mobile phase, ACN, MeOH or THF. Owing to the huge differences in the solvent characteristics, the choice of different mobile phase usually results in different selectivity, which could therefore meet most of the separation requirements.

Experimental

To compare the selectivity of different solvents as the mobile phase, ACN, MeOH and THF was used as the mobile phase for the flash purification of the sample mixture (as shown in Figure 2) by a preparative flash chromatography system. The experimental parameters were summarized in Table 1. The chromatograms were shown as Figure 3 to Figure 5.
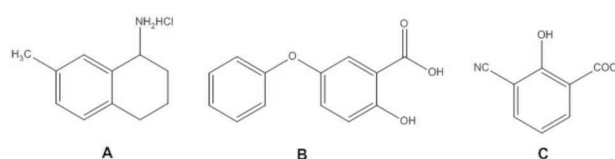
**Figure 2. The chemical structure of the Sample A, B and C.**

Table 1. The experimental parameters.

| Instrument	A preparative flash chromatography system					
Cartridges	25g SepaFlash™ C18 flash cartridge (40-63µm, 90Å, Order number: SW-5211-025-IR)					
Wavelength	220 nm (detection), 254 nm (monitoring)					
Sample	60mg mixture of Sample A (20mg), B (20mg) and C (20mg), dissolved in 2mL MeOH and injected into cartridge by an injector					
Mobile phase	Solvent A: 0.1% TFA in water Solvent B: (1) 0.1% TFA in ACN; (2) 0.1% TFA in MeOH; (3) 0.1% TFA in THF					
Flow rate	15 ml/min					
Gradient	ACN-water system		MeOH-water system		THF-water system	
	B(%)	Time (min)	B(%)	Time (min)	B(%)	Time (min)
	15	0	15	0	15	0
	15	3.5	15	3.5	15	3.5
	36	6.0	36	6.0	36	6.0
	36	8.0	36	8.0	36	7.5
	60	10.5	60	10.5	60	10.0
	60	13.5	60	13.5	60	13.0
	85	18.0	85	20.0	74	15.0
			85	22.0		

Results and Discussion

As we can see from the chromatograms (Figure 3 – Figure 5), differences in selectivity exist in the practical separation procedure for these three most popular organic solvents. For the benzoic acid derivatives (Sample B and Sample C), the eluting effect by these three solvents are not equivalent. Comparing with MeOH, ACN and THF show more

eluting power, resulting in less retention time for the sample mixtures. However, the separation effect of Sample B and Sample C for THF was not as satisfactory as other two solvents. Furthermore, regarding organic hydrochloride compounds (Sample A), good peak resolution and appropriate retention time could be obtained while choosing MeOH as the eluting solvent. In contrast, barely retention time was observed for THF (as shown in Figure 5), while splitting peaks appeared for ACN (as shown in Figure 3).
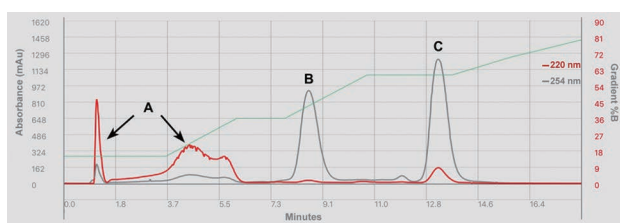
Figure 3. The chromatogram of the sample mixture when using ACN-water as the mobile phase.
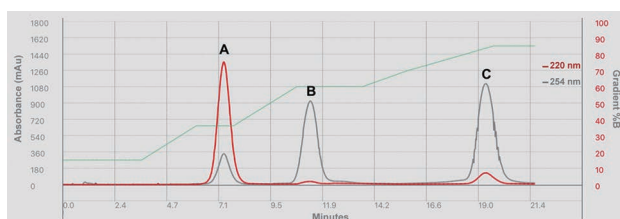
Figure 4. The chromatogram of the sample mixture when using MeOH-water as the mobile phase.
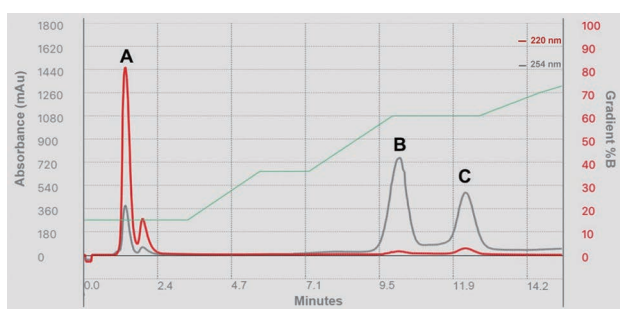
Figure 5. The chromatogram of the sample mixture when using THF-water as the mobile phase.

Conclusion

In conclusion, appropriate solvent should be chosen as the mobile phase for reversed-phase separation. There are several items should be noticed while choosing eluting solvents, which is described in detail as follows.

•The solvent with smaller viscosity should be chosen since the mobile phase with too high viscosity will result in system overpressure especially when flash column packed with small particle sized materials is used.

•The chosen solvent should have a good solubility for the sample to be separated, otherwise the separation effect as well as the peak shape will be compromised.

•Considering the problem of UV absorbance of solvent, the solvent without absorbance at the detection wavelength should be selected. For example, THF should be replaced by ACN or MeOH since THF has absorbance problem at UV range.

•In general, ACN-water system is recommended as the preferred eluting solvent. MeOH is the second recommended solvent. And THF is the final option when ACN or MeOH is not the suitable solvent.

References

(1) J.W. Dolan, LCGC North America 28 (12), 1022-1027 (2010).