Toward Development of "Generic" Separation Methods for Achiral Pharmaceutical Analysis Using SFC

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INTRODUCTION

In the 1990s, the pace of drug discovery accelerated rapidly as screening and chemical synthesis transitioned from the traditional relatively linear iterative process to parallel approaches. High-speed parallel synthesis created the need for rapid analysis and screening by HPLC. Beginning about 1996, the use of reversed phase gradients on C_{18} media in short column formats as 'generic separation methods' developed in critical applications such as analysis of crude synthetic isolates, in-vitro drug metabolism assays, and purification of drug discovery leads.

Might normal phase chromatography, HPLC and SFC, be adapted to allow their use with 'generic' separation methods? What are the characteristics of a successful 'generic' method? What hurdles must be overcome if SFC is to be as generally useful as RP-HPLC?

It is worth remembering that a generic separation method is fundamentally different from an optimized method. Generic chromatography is a practical art. Definitions of good chromatography and analytical figures of merit largely do not apply. Instead, *the only valid measure of good is whether the job at hand gets done* – is the desired compound distinct within the chromatogram?

Table 1. Attributes of Generic Gradients & Enabling Technologies

Attribute	Enabling Technology
Retention and elution of most compounds of interest: High peak capacity	Gradient elution HPLC solid phase characteristics: – Small particles, high phase loading – End-capping – pH and water-stable phases
Universal detection	Diode array UV detection Mass Spectrometry (MS) detection Evaporative Light Scattering Detection (ELSD) Chemiluminescent Nitrogen Detection (CLND)
Differential (specific) detection	Diode array UV detection MS and MS/MS detection
Rapid chromatographic cycle	Short, efficient columns Alternating column regeneration Low dwell volume HPLC Ultra High Pressure LC (UPLC)

Without the development of three primary technologies, gradient HPLC would not have developed as a generic analytical approach:

- Generally retentive phases, such as C_{18}
- Atmospheric pressure (API) mass spectrometric detection
- Short, high capacity HPLC columns

TESTING COLUMN SELECTIVITY

Using a mixture of drugs and drug-like like compounds as a test standard, we examined a variety of stationary phases for compound retention and elution.

Table 2. Drug-Like Compounds Used in the Test Standard

Compound (elution order on 2-EP)	Name	
1	Citral	
2	Lidocaine	
3	Caffeine	
4	Ibuprofen	
5	Amitriptyline	
6	Propranolol	
7	Procainamide	
8	Sulpiride	
9	b-D-Arabinofuranosyl-cytosine	
10	Sulfrinpyrazone	

The test standard was injected onto each column using an SFC gradient method of 5%-65% cosolvent in CO_2 (total flow of 2.0 mL/min) over 5 or 12.5 minutes, (depending on column length) followed with a 10 second hold at 65% and a return to initial condition. The mobile phase cosolvent is 50:50 methanol:isopropanol with 0.1% diethylamine (MeOH:IPA 0.1%DEA).

Table 3. Initial Selection of Study Columns Used With Standard Elution Method (Figures 1a and 1b)

Column	Description
2-EP	2-Ethylpyridine 5µ, 4.6x100mm, Princeton Chromatography
EP	Ethylpyridine SFC 5µ, 4.6x100mm, ES Industries
CN	PrincetonSFC CN, 5µ, 4.6x100mm, Princeton Chromatography
Diol	PrincetonSFC Diol, 5µ, 4.6x100mm, Princeton Chromatography
NO2	Epic-NO2 SFC, 5µ, 4.6x100mm, ES Industries
Pyr-Am	Pyridyl Amide SFC, 5µ, 4.6x100mm, ES Industries
Silica	PrincetonSFC Silica, 5µ, 4.6x100mm, Princeton Chromatography

Column	Description
YMC-Diol	YMC-Pack Diol-120-NP, 4.6 x 250mm, 5um, YMC
YMC-CN	YMC-Pack CN, 4.6 x 250mm, 5um, YMC
NH2	YMC-Pack NH2, 4.6 x 250mm, 5um, YMC
SIL	YMC-Pack SIL, 4.6 x 250mm, 5um, YMC
PVA-SIL	YMC-Pack PVA-SIL-NP, 4.6 x 250mm, 5um, YMC
Polyamine II	YMC-Pack PolyAmine II, 4.6 x 250mm, 5um, YMC

Figure 1a. Column Selectivity Study Chromatograms (10 cm, 8 minute gradient)

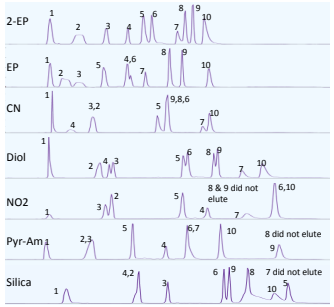
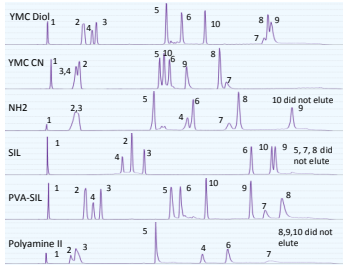


Figure 1b. Column Selectivity Study Chromatograms (25 cm, 17 minute gradient)



Unmodified silica is a poor choice for generic chromatography due to its stability and reactivity. The bonded phases show large differences in selectivity, offering a range of alternatives. It is also worth noting that a number of columns continued to retain selected compounds beyond the end of the gradient at 65% cosolvent. Because higher cosolvent flow rates are impractical in SFC, these columns may be less generally suitable.

SOLVENT SELECTIVITY

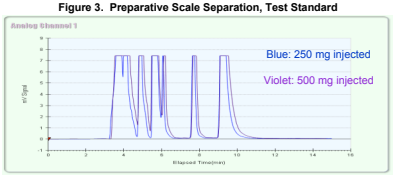
We chose to study solvent selectivity using only the 2-EP column. We note substantial differences in selectivity of the column across the solvent series MeOH/50:50/IPA. However, despite changes in elution order all the solvent systems meet the basic criteria of an adequate generic separation method.

Figure 2. Solvent Selectivity Study Chromatograms

PREPARATIVE GENERIC GRADIENT SFC

Figure 3 shows the result of loading a five compound mixture (procainamide, sulpiride, amitryptaline, lidocaine, caffeine) on a 3.0 x 25.0 cm 5µ 2-EP column. The gradient elution (5-50% 50:50 MeOH:IPA, 1% DEA, 80 g/min) results in tight chromatographic bands and adequate separation for preparative chromatography.

Figure 3. Preparative Scale Separation, Test Standard



CONCLUSIONS

We have investigated the performance of several stationary phases and solvent systems and conclude that generic normal phase chromatographic methods may be developed using an approach similar to the development path of generic RP-HPLC gradient chromatography. The approach may be adapted successfully to larger scales.

SELECTED REFERENCES

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