INTRODUCTION
In the 1980s, 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 C18 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.

Reversed-phase gradient HPLC is now frequently used for purification. A common approach is to inject up to 100 mg of crude syntheses product on a 20 x 50 mm C8 column and elute with a <10 minute single-step wash of water:methanol:acetonitrile, usually with an organic acid or buffer modifier. MS detection (or UV detection after LCMS analysis) ensures that the desired peak is collected. A disadvantage is the slow and warm dry-down of fractions necessitated by the aqueous media.

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?

DEFINING ‘GENERIC’ GRADIENTS
An optimized method disperses analytes and artifacts into separate bands that elute with predictable profile and retention time and evoke a response at a detectable proportional to the quantity of compound. A generic method is used without optimization—every analyte or mixture is applied to the column with regard to whether the detector will respond or not, without consideration of whether mixture components will co-elute. Time constraints make traditional method optimization impossible; but more importantly, compound-specific optimization is not desirable; efficient drug discovery aims to understand structure-activity relationships across large sets of compounds, and this is simplest when each handled identically.

Generic chromatography is a practical art. Definitions of good chromatography—elution order figures of merit used to evaluate peak shape and tailing and selectivity—might be redefined by the strength of an optimized method—largely do not apply. Instead, the only valid measure of good is whether the job at hand gets done—a desired compound is found within the sample injected?

Table 1. Attributes of Generic Gradients & Enabling Technologies

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Enabling Technology</th>
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| Retention and elution of most compounds of interest: High peak capacity | Gradient elution with solvents:
- High selectivity
- High efficiency
- Low gradient ramping
- Low solvent series

Universal detection | Single-mode UV detection
- Single-mode fluorescence detection
- Low wavelength detection

Differential (specific) detection | Single-mode UV detection
- Single-mode fluorescence detection
- Low wavelength detection

Rapid chromatographic cycle | Short, efficient column
- Fast, powerful gradient
- High efficiency
- Rapid column regeneration
- High yield

Without the development of three primary technologies, gradient HPLC could not have developed as a generic analytical approach: gradients, gradient optimization, and solvent series.

In other words, it was necessary to demonstrate that the majority compounds of functional interest could be retained and eluted from the same column, and that one could detune the single component of interest selectively. It was also necessary to do the analysis rapidly enough that large compound sets could be analyzed; as the pressure to develop generic methods was coming from the parallel chemistry movement.

Prior to 1997, few columns were available with small particle size and in shorter formats. The ubiquity of rapid generic gradients with RP-HPLC drove the development of columns, phases, and systems technology rapidly over the last 10 years. The liquid-phase development of RP-HPLC over normal phase and SFC in generic gradient applications partially due to the fact that ES/MS sources, until recently, required a mixed aqueous solvent to generate [M+H]+ ions. Recent developments in RP-HPLC API MS sources have enhanced sensitivity and allow analytes to be ionized successfully from virtually any mixed solvent system containing a proton donor (e.g. hexane, acetonitrile, CO2, methanol). Recently, commercially available SFC/MS systems have demonstrated not only easy coupling but highly stable and reliable ionization of pharmaceutical compounds.

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

In evaluating the chromatograms, we are interested in whether all compounds are retained and elute, a necessity in a generic method. We look not for broadened or tailing peaks, or an indication of incomplete transfer to the stationary phase and of inefficient mass loading. Loading is critical if preparative chromatographic methods are to be developed.

Figure 1. Column Selectivity Study Chromatograms

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

<table>
<thead>
<tr>
<th>Compound (elution order on 2-EP)</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Citral</td>
</tr>
<tr>
<td>2</td>
<td>Lidocone</td>
</tr>
<tr>
<td>3</td>
<td>Caffeine</td>
</tr>
<tr>
<td>4</td>
<td>Epibrocine</td>
</tr>
<tr>
<td>5</td>
<td>Ambiproline</td>
</tr>
<tr>
<td>6</td>
<td>Propranolol</td>
</tr>
<tr>
<td>7</td>
<td>Propanolamide</td>
</tr>
<tr>
<td>8</td>
<td>Sulpiride</td>
</tr>
<tr>
<td>9</td>
<td>b-D-Arabinofuranosil-cytosine</td>
</tr>
<tr>
<td>10</td>
<td>Sulfinpyrazone</td>
</tr>
</tbody>
</table>

Table 3. Initial Selection of Study Columns Used With Standard Elution Method

<table>
<thead>
<tr>
<th>Column</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-EP</td>
<td>2-Ethylxyline So, 4.6x100 mm, Princon Chromatography</td>
</tr>
<tr>
<td>EP</td>
<td>Chromatography Epoxide SFC So, 4.6x100 mm, ES Industries</td>
</tr>
<tr>
<td>CN</td>
<td>Chromatography CN, So, 4.6x100 mm, ES Industries</td>
</tr>
<tr>
<td>Dil</td>
<td>Deltanorl SFC Dil, 4.6x100 mm, Princon Chromatography</td>
</tr>
<tr>
<td>NO5</td>
<td>Chromatography Epoxide SFC So, 4.6x100 mm, ES Industries</td>
</tr>
<tr>
<td>PAM</td>
<td>Chromatography PAM Elute So, 4.6x100 mm, ES Industries</td>
</tr>
<tr>
<td>SFC</td>
<td>Chromatography SFC Siv, 4.6x100 mm, Princon Chromatography</td>
</tr>
</tbody>
</table>

The test standard was injected onto each column using an SFC gradient method of 5%-65% co-solvent in CO2 (total flow of 2.0 mL/min) over 5 minutes, followed by a 10 second hold at 65% and return to initial condition. The chromatograms shown in Figure 1 are presented on an 8 minute x-axis. The mobile phase consisted of 50:50 methanol:isopropanol with 0.1% diethylamine (MachEl/0.1% DEA).

Although 2-EP has a reputation for producing better peak shape with moderately basic analytes than other (less basic) bonded phases, the co-solvent with no organic base modifier allows substantial tailing with propranolol, procainamide, and sulfinpyrazone. We have no estimate of these compounds pKa values in mixed organic solvent, but believe the tailing is related to their basicity.

We note substantial differences in selectivity of the columns across the solvent series MeOH50:50/IPA. However, despite changes in elution order the solvent systems meet the basic criteria of an adequate generic separation method.

Note that in the applications of generic RP-HPLC, chromatographers frequently screen unknown mixtures using two methods in which the same column is used with two solvent systems offering different selectivity. For example, analytes may be retained on CN using a water:acetonitrile:triacetate bicarbonate buffer, while other compounds are better retained and resolved using water:acetonitrile:formic acid. We suggest that a similar approach, a standard column run with two methods offering differentanalyte selectivity, might be a useful approach with generic SFC.

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 mm 5-µm 2-EP SFC column. The gradient elution is 50:50 MeOH:IPA, 1% DEA, 20 g/min results in tight chromatographic bands and adequate separation for preparative chromatography. The injection was separated into the column stream so that the sample was introduced to the column headstream at the initial gradient condition of low solvent strength. The violet trace represents an injection of 500 mg of the mixture (equal masses each compound) on column.

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.

Generic gradient RP-HPLC became a valuable methodology when several technological milestones were achieved: LC-MS coupling, stable stationary phases, and sufficient preparative chromatography systems. These developments in SFC are in earlier stages of development, but viable and suggest promising bright spot.

SELECTED REFERENCES
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