Faster and improved HPLC separations within pharmacopoeia guidelines

**Introduction**

Keeping the cost of generic drugs affordable, while ensuring their purity and efficacy is a worthy goal. In this study, we demonstrate an efficient way to significantly reduce the cost of analyzing six blockbuster generic drugs by varying column dimensions within the allowed limits of United States (USP) and European Pharmacopoeia (EP). USP and EP provide recommendations for column dimensions and packing materials along with guidelines clearly stating, how far you may deviate from these column parameters. If the deviations are within the allowed limit there is no need to revalidate the method, but system suitability criteria must be met.

Reduction in cost of analysis using current USP guidelines for gradient analysis was demonstrated using atorvastatin, olanzapine, fluticasone propionate and risperidone. These sets of experiments include binary and ternary gradient methods on C8 and C18 packing materials. USP is under discussion to relax the current restrictions on particle size for isocratic separations. Using tramacol as an example, time and solvent reduction for isocratic analysis is demonstrated based on USP Interim Revision Announcements (IRAs).

Cost reduction by modifying column dimensions within EP guidelines was demonstrated using salmeterol xinafoate.

**Experimental**

![Image of Agilent equipment](image)

**Figure 1: Agilent 1290 Infinity LC with Intelligent System Emulation Technology (ISET).** The ISET emulation algorithm delivers identical gradient mixing conditions as selected other instruments and gives matching chromatographic resolution.

**Procedure**

- Purity/assay methods of the drugs were performed using USP/EP pharmacopeia recommendations.
- Then selected three (two only for tramacol) smaller column dimensions for each of the analytes which were within USP/EP allowed deviation limit.
- New gradient parameters were derived using Agilent automated Method Translator and Cost Saving Calculator tool.
- To verify acceptable performance, system suitability testing of each analytes for each column dimension was performed.

Using ISET, the Agilent 1290 Infinity LC was easily emulated from one to another instrument model with respect to the column dimension used for the analysis. However, the use of narrow bore columns may demand Ultra High Pressure LC (UHPLC) systems with low delay and dispersion volumes. Agilent 1290 infinity LC offers the best in class power range for this purpose.

The cost of analysis was calculated for all the experimental conditions.

**Allowed column deviations**

<table>
<thead>
<tr>
<th>Column Parameter</th>
<th>USP limit for deviation</th>
<th>EP limit for deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length</td>
<td>≥ 70%</td>
<td>≥ 70%</td>
</tr>
<tr>
<td>Internal diameter</td>
<td>≥ 30% 25%</td>
<td>≥ 25%</td>
</tr>
<tr>
<td>Particle size</td>
<td>≥ 50%</td>
<td>≥ 50%</td>
</tr>
</tbody>
</table>

Table 1: Allowed column deviations as per USP <821> and EP recommendations

**Results and Discussion**

The excellent separation of system suitability mix was observed for all cases. The use of smaller length columns reduced analysis time and decreased solvent consumption. The smaller particle sizes in the columns helped to enhance the resolution of peaks. The chromatograms corresponding to all conditions for the drugs with cost of analysis are given in the following figures.

1. **Atorvastatin (USP Method, DAD Signal 244nm)**

   *Sample: 0.05 mg/mL of USP Atorvastatin Calcium and Related Compound (RCC)*

   **Figure 1:** Agilent 1290 Infinity LC

   **Figure 2:** Separation of atorvastatin system suitability mix using USP and newly developed cost effective methods

2. **Olanzapine (USP Method, DAD Signal 229nm)**

   *Sample: 3µg/mL of each (1)Olanzapine RCC and (2)Olanzapine RCC and 25µg/mL of (3)Olanzapine,

   **Figure 3:** Separation of olanzapine system suitability mix using USP and newly developed cost effective methods

3. **Fluticasone (USP Method, DAD Signal 238nm)**

   *Sample: 0.2mg/mL of Fluticasone Propionate USP System Suitability Mix Contents: 1)RCC, 2)RCC, 3) RCD, 4) API, 5) RBC

   **Figure 4:** Separation of fluticasone system suitability mix using USP and newly developed cost effective methods

**Conclusions**

- A simple and acceptable approach to reduce the total cost of analysis for generic drugs is demonstrated.
- By this approach the cost of analysis can be reduced up to 70%.
- This study has demonstrated cost reduction in various methods including binary, ternary, isocratic and gradients.
- *No compound mixed*

**References**

2. Agilent publications: SRI-1521EN, SRI-1526EN, SRI-1529EN, SRI-1535EN, SRI-6312EN.