Faster and improved HPLC separations within pharmacopeia guidelines

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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 Pharmacopeia (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 tramadol as an example, time and solvent reduction for isocratic analysis is demonstrated based on USP Interim Revision Announcements (IRAs) [1].

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

Experimental

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.

Instrumentation

Agilent 1290 Infinity LC system with ISET technology which consists of the following modules was used for the experimentation,

- Agilent 1290 Infinity Binary Pump
- Agilent 1290 Infinity High Performance Autosampler
- Agilent 1290 Infinity Thermostatted Column Compartment
- Agilent 1290 Infinity Diode Array Detector with Max-Light flow cell
- Software used was Agilent OpenLAB CDS ChemStation.

Procedure

- Purity/assay methods of the drugs were performed using USP/EP pharmacopeia recommendations. [2]
- Then selected three (two only for tramadol) 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 four experimental conditions.

Allowed column deviations

Column Parameter	USP limit for deviation[7]	EP limit for deviation [9]
Length	± 70%	± 70%
Internal diameter	No limit, but keep constant linear velocity	± 25%
Particle size	- 50%	- 50%

Table: 1 Allowed column deviations as per USP <621> 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 experiment conditions for all 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 (RC)B.

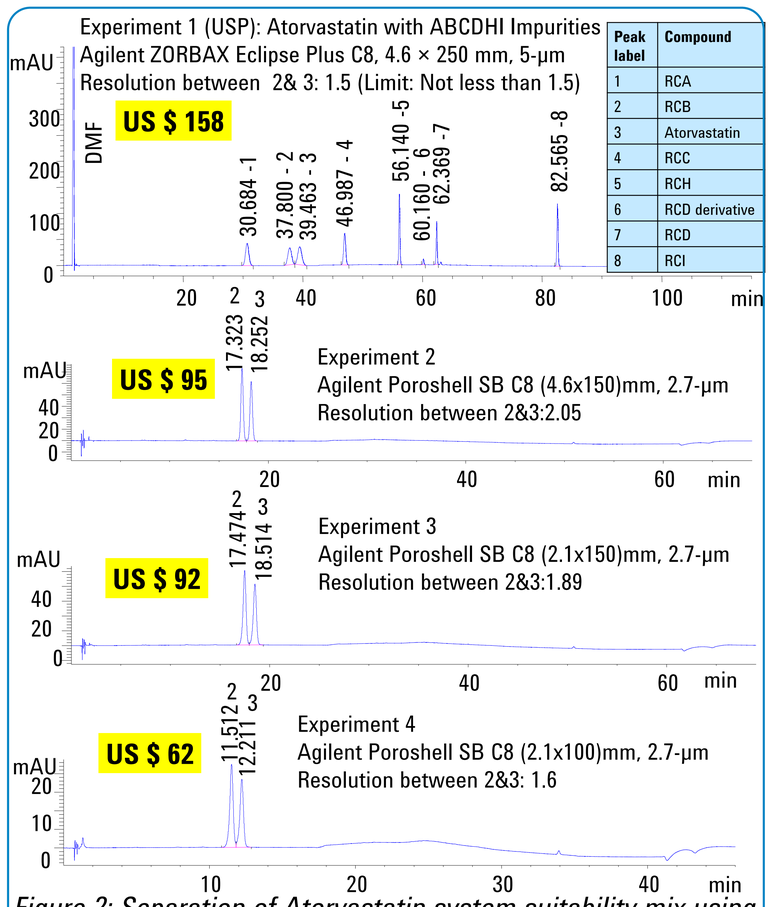

Figure 2: Separation of Atorvastatin system suitability mix using USP and newly developed cost effective methods

2.Olanzapine (USP Method, DAD Signal 220nm)

Sample: 2µg/mL each of (1)Olanzapine RCB and (2)Olanzapine RCC and 20µg/mL of (3)Olanzapine, .

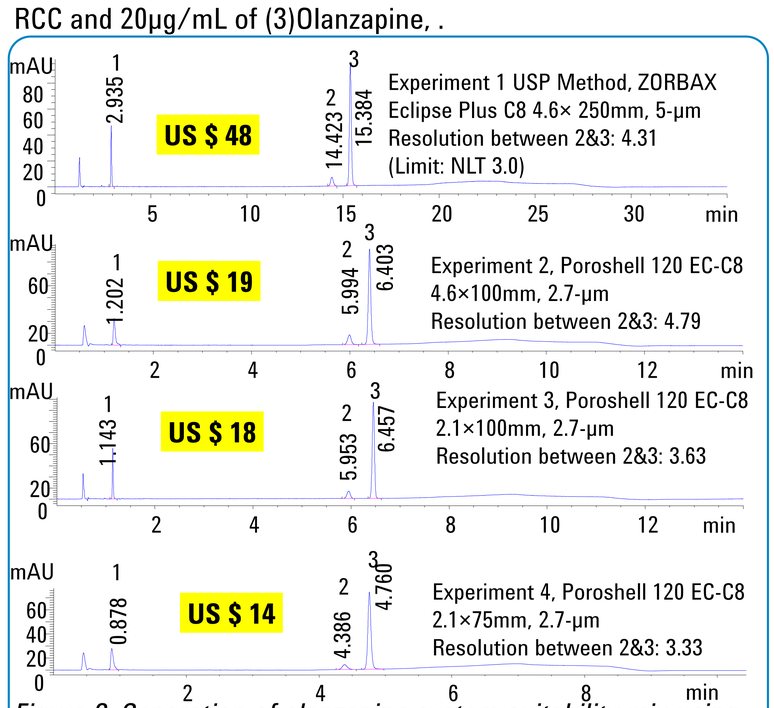

Figure 3: Separation of olanzapine system suitability mix using USP and newly developed cost effective methods

3. Fluticasone (USP Method, DAD Signal 239nm)

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

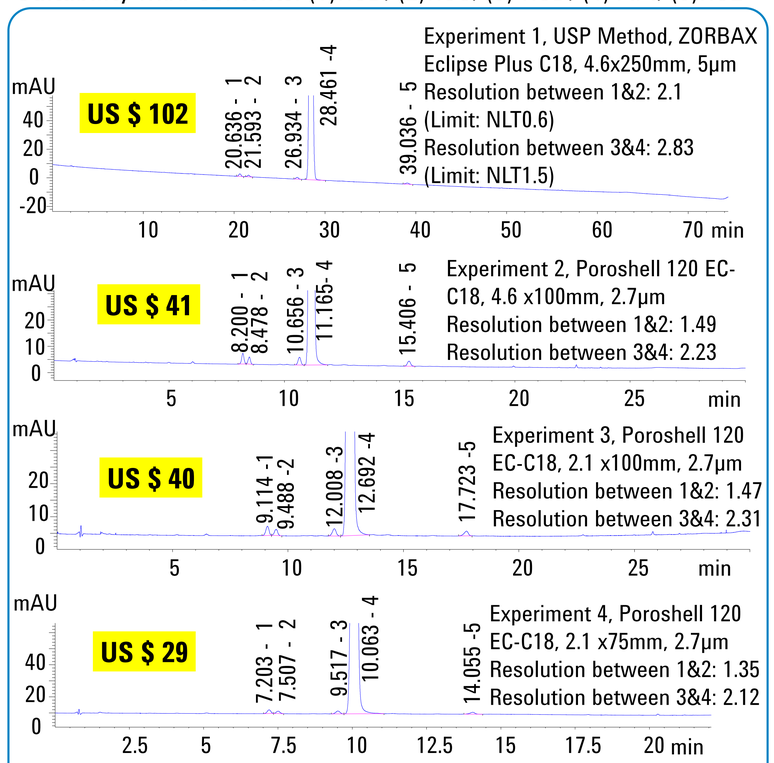

Figure 4: Separation of fluticasone system suitability mix using USP and newly developed cost effective methods

Results and Discussion

4. Risperidone (USP Method, DAD Signal 275nm)

Sample: 1 mg/mL USP Risperidone System Suitability Mixture. Contents: (1)Z-Oxime, (2)9-Hydroxyrisperidone, (3)Risperidone, (4)6-Methylrisperidone

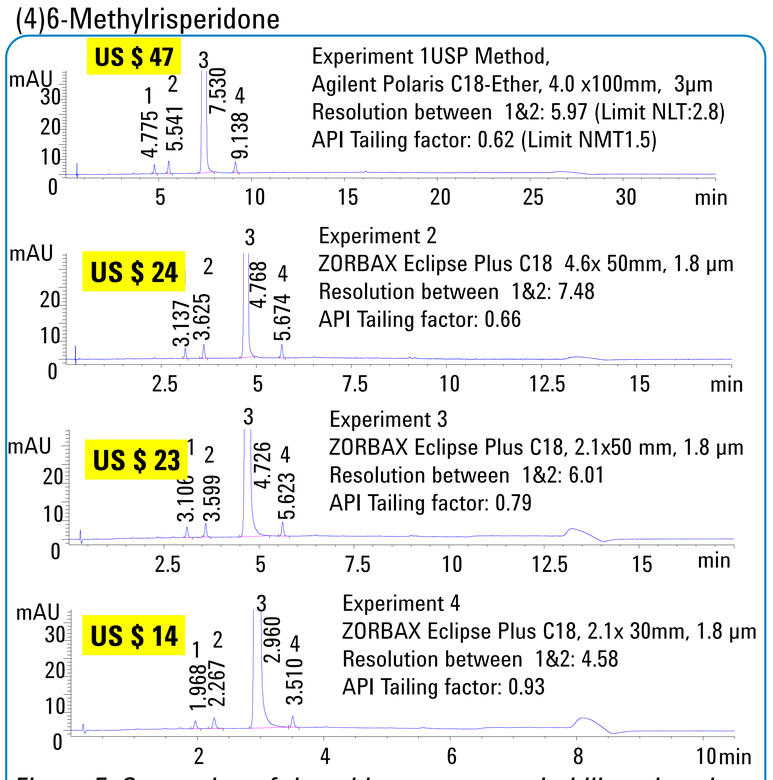

Figure 5: Separation of risperidone system suitability mix using USP and newly developed cost effective methods

5. Tramadol (USP Method, DAD Signal 270nm)

Sample: 0.05 mg/mL each of (1)Tramadol HCl and (2)RCA

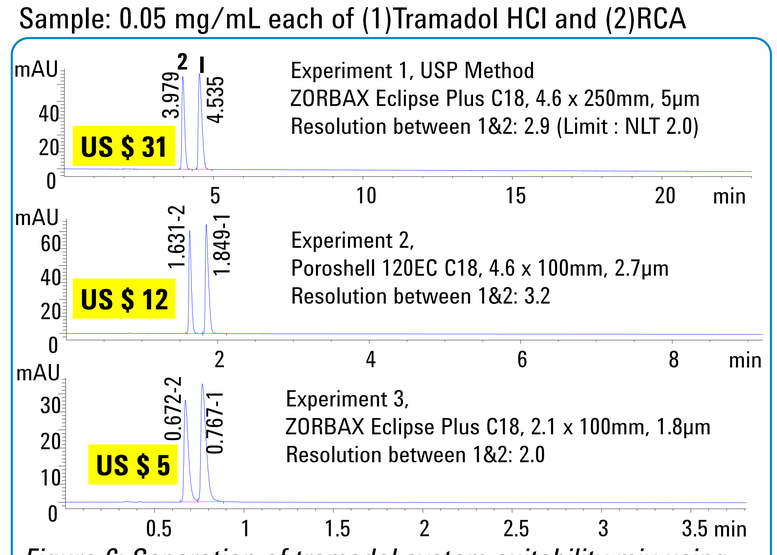

Figure 6: Separation of tramadol system suitability mix using USP and newly developed cost effective methods

6. Salmeterol Xinafoate (EP Method, DAD Signal 278nm)

Sample: 5.5mg/mL of salmeterol xinafoate system suitability mix containing (1) API, (2) impurities E and (3)G

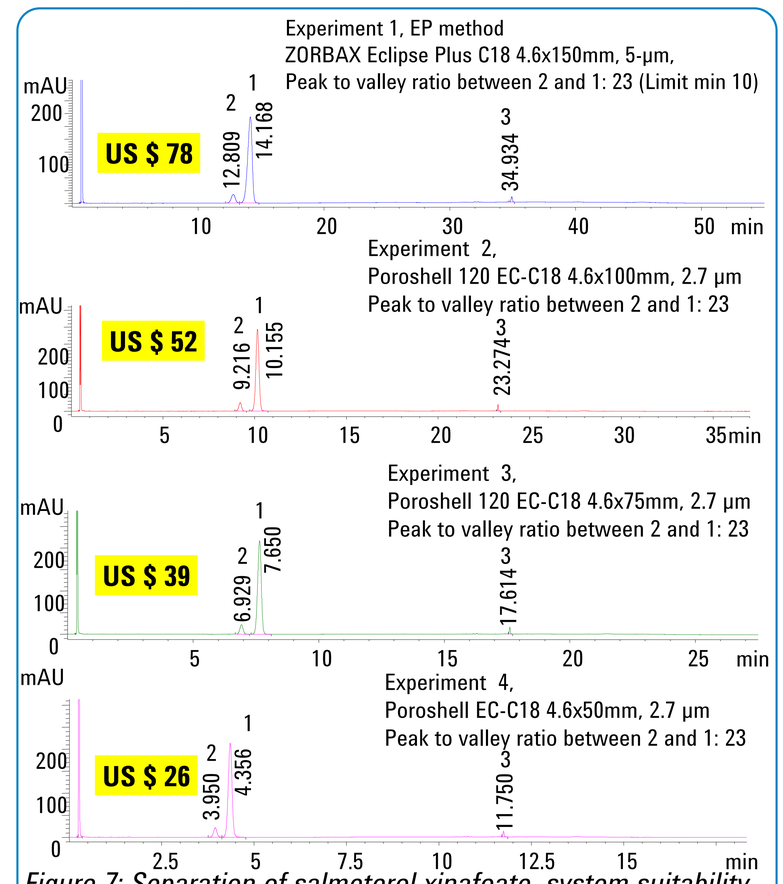

Figure 7: Separation of salmeterol xinafoate system suitability mix using USP and newly developed cost effective methods.

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 missed"

References

1. *USP Pharmacopeial Forum (PF) 38(2) In-Process Revision: <621> chromatography (USP36-NF31 1S), Page 17, 2012.*
2. *Agilent publications, 5991-1053EN, 5991-0278EN, 5991-1602EN, 5991-0396EN, 5991-0733EN and 5991-0394EN.*