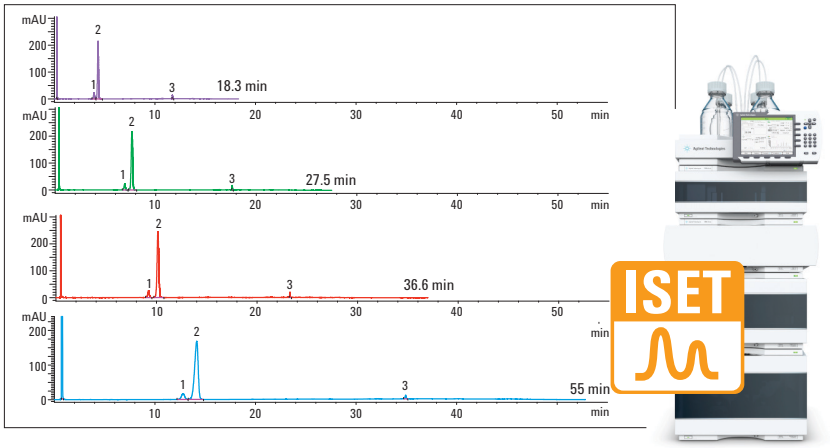
Developing faster methods for generic drugs within EP 2.2.46E allowed limits

Higher throughput and cost reduction for salmeterol xinafoate analysis using the Agilent 1290 Infinity LC System with ISET

Application Note

Pharmaceutical QA/QC



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Abstract

This Application Note describes an approach to reduce cost per analysis and increase throughput by varying column dimensions using the European Pharmacopeia (EP) method of chromatographic purity for salmeterol xinafoate as an example. Cost reduction and higher throughput was made possible by transferring the EP method into three shorter gradients using Agilent Poroshell 120 EC C18 columns of various lengths within the allowed EP limit. System suitability testing was carried out with varied column dimensions to verify the acceptable performance of the liquid chromatographic (LC) system to perform the purity analysis. The Agilent 1290 Infinity LC System with Intelligent System Emulation Technology (ISET) was used to ensure identical gradient mixing conditions of various other HPLC systems configurations according to the column dimensions. The results show that approximately 67% in time and solvent can be saved as compared to the original pharmacopeia method.

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Introduction

Salmeterol is a long-acting beta 2-adrenergic receptor agonist drug and usually prescribed for the treatment of asthma and other respiratory diseases[1]. The substance is one of the best selling block buster drugs on the global pharma market. The EP method of analysis for chromatographic purity takes 55 minutes and uses a 150 × 4.6 mm column with 5-µm octadecylsilyl silica packing[2]. Assuming that a cost of US $ 60/L for acetonitrile, a cost of US $ 1.5/L for solvent disposal and the cost of running the HPLC system as US $ 80/hour, the total cost of running the EP method for salmeterol xinafoate can be calculated as US $ 78.2. One can vary the column dimensions, which, in turn, helps reduce the cost per analysis by reducing solvent consumption and cycle time. There are general guidelines from EP which state the permitted deviations for these column parameters. If the modifications are made within the allowed pharmacopeia limit, the need for method revalidation can be eliminated. The EP guideline on permitted column dimension deviations for LC methods is given in Table 1[3].

In this Application Note, we show a significant reduction in analysis cost by using three different smaller column dimensions within the allowed deviations. System suitability testing was performed and calculated the cost savings in gradient time and solvent consumption. The use of smaller length columns ensured the reduction in analysis time with decreased solvent consumption, and smaller particle sizes promised enhanced resolution of peaks. Using smaller particle-sized columns may require LC systems with higher pressure withstanding capabilities. Chances of obtaining a dissimilar elution profile with different instruments models are high here due to the difference in instrument delay volumes. Agilent 1290 Infinity LC Systems operated with ISET can minimize this problem by emulating identical gradient mixing conditions for different instruments. ISET ensures the uncompromised performance of a 1290 Infinity LC System as a universal LC system.

Column parameter	EP limit for deviation
Length	± 70%
Internal diameter	± 25%
Particle size	– 50%

Table 1
Allowed column deviations as per EP 2.2.46E recommendation.

Experimental

Instruments

The Agilent 1290 Infinity LC System included the following modules:

- Agilent 1290 Infinity Binary Pump with integrated vacuum degasser (G4220 A) and 35-µL Jet Weaver mixer.
- Agilent 1290 Infinity High Performance Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A) with Max-Light flow cell (1.0 µL dispersion volume, 10 mm path length) (G4212-60008)

Software

- Agilent ChemStation C.01.03

The EP method was performed as Experiment 1 using an Agilent ZORBAX Eclipse Plus C18 4.6 × 150 mm, 5-µm column which is the pharmacopeia recommended column dimension. Later, three different column dimensions were selected and performed as Experiments 2, 3, and 4. For Experiment 2, an Agilent Poroshell 120 EC-C18 4.6 × 100 mm, 2.7-µm column (length approximately 2/3 of the original length, same internal diameter but smaller particle size) was selected. For Experiment 3, an Agilent Poroshell 120 EC-C18 4.6 × 75 mm, 2.7-µm column (length approximately 1/2 of the original length, same internal diameter but smaller particle size) was selected, and for Experiment 4, an Agilent Poroshell EC-C18 4.6 × 50 mm, 2.7-µm column (minimum length according to EP limits, same internal diameter and smaller particle size) was selected. The details of the other columns dimensions and observed deviations for all the four experiments are tabulated in Table 2.

Reagents and materials

The commercially available EP reference standards for salmeterol xinafoate system suitability mix contain impurities E and G. Acetonitrile was of super gradient grade and was purchased from Lab-Scan (Bangkok, Thailand). Highly purified water from a Milli Q water purification system (Millipore Elix 10 model, USA) was used for the experiment. Other chemicals like ammonium acetate, sodium dodecyl sulphate, and glacial acetic acid were purchased from Aldrich (India).

Column parameter	EP recommendation	Experiment 1		Experiment 2		Experiment 3		Experiment 4	
		Actual	% Deviation	Actual	% Deviation	Actual	% Deviation	Actual	% Deviation
length	150 mm	150 mm	0	100 mm	–33.3	75 mm	–50	50 mm	–66.6
Particle size	5 µm	5 µm	0	2.7 µm	–46	2.7 µm	–46	2.7 µm	–46

Table 2
Various column dimensions percentage deviations used for the experiments.

Chromatographic parameters

The buffers and mobile phases were prepared as per the pharmacopeia method. The details of the buffers, mobile phases and diluent preparation used for this experiment are given in Table 3. The detection at 278 nm was used. Since the internal diameter for all columns were kept constant, a constant flow rate of 2 mL/min was used for all trials. The analysis using this EP recommended column dimension (Experiment 1) was carried out using the Agilent 1290 Infinity Binary System with ISET emulating to Agilent 1100 Series Binary Pump. For Experiments 2, 3, and 4, the 1290 Infinity LC System was used to emulate the 1260 Infinity Binary Pump (pressure limit 600 bar). The detailed chromatographic method parameters for each experiment are tabulated in Table 4.

Procedure

The system suitability sample was prepared as per EP recommendation using the salmeterol xinafoate system suitability mix containing impurities E and G (11 mg of the standard mix dissolved in 2 mL diluent). The method transfers for all the experiments were carried out using the Agilent Method Translator (V: 2) in "Simple conversion" mode. System suitability testing for related substance as per EP method was performed using all four experiment conditions.

The first criteria for the system suitability test for the salmeterol related substance is to measure peak-to-valley ratio (Hp/Hv) between impurity E and the salmeterol main peak. The limit is a minimum of 10, where Hp = height above the baseline of the impurity E peak and Hv = height above the baseline of the lowest point of the curve separating this peak from the salmeterol peak. The similarity of the resulting chromatogram with the chromatogram supplied with the standard system suitability mix is the second criteria. To verify the precision and accuracy, relative standards deviations (RSD) of retention time (RT) and area was measured.

Buffer	Details
Solution A	7.71 g/L solution of ammonium acetate
Solution B	28.84 g/L solution of sodium dodecyl sulphate , adjust the pH to 2.7 with glacial acetic acid
Mobile phase A	Solution A: Solution B: Acetonitrile; 24:24:52
Mobile phase B	Acetonitrile
Diluent	Acetonitrile: Water : 50:50

Table 3
Buffers, mobile phases and diluent as per EP method.

	Agilent 1290 Infinity Binary LC System with ISET			
Parameter	Experiment 1 Emulated as Agilent 1100 Series LC	Experiment 2 Emulated as Agilent 1260 Infinity LC	Experiment 3 Emulated as Agilent 1260 Infinity LC	Experiment 4 Emulated as Agilent 1260 Infinity LC
Injection volume	10 µL	7 µL	5 µL	3.5 µL
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm	Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm	Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm	Agilent Poroshell 120 EC-C8, 4.6 × 50 mm, 2.7 µm
Gradient	At 0 min 0% B At 16 min 0% B At 36 min 70% B At 45 min 70% B At 50 min 0% B At 55 min 0% B	At 0 min 0% B At 10.67 min 0% B At 24 min 70% B At 30 min 70% B At 33.33 min 0% B At 36.67 min 0% B	At 0 min 0% B At 8 min 0% B At 18 min 70% B At 22.5 min 70% B At 25 min 0% B At 27.5 min 0% B	At 0 min 0% B At 5.33 min 0% B At 12 min 70% B At 15 min 70% B At 16.67 min 0% B At 18.33 min 0% B
Acquisition rate	5 Hz	20 Hz	20 Hz	20 Hz

Table 4
Detailed chromatographic parameters for all the four experiments.

Results and discussion

Separation and detection

The peaks are well separated in all four experimental conditions (Figure 1). The system suitability results obtained from all the four experiment conditions are tabulated in Table 5. They are within the acceptance criteria even under Experiment 4 (Poroshell EC-C18 4.6 × 50 mm, 2.7-µm column) condition. The peak-to-valley ratio between impurity E and salmeterol was one of the critical parameters to be verified for the system suitability test. This value was higher for all three new methods compared to the value obtained with the original pharmacopeia method. The match of the system suitability chromatogram obtained from all trials with the standard chromatogram was established by evaluating the relative retention time (RRT) of impurity E and the salmeterol peak.

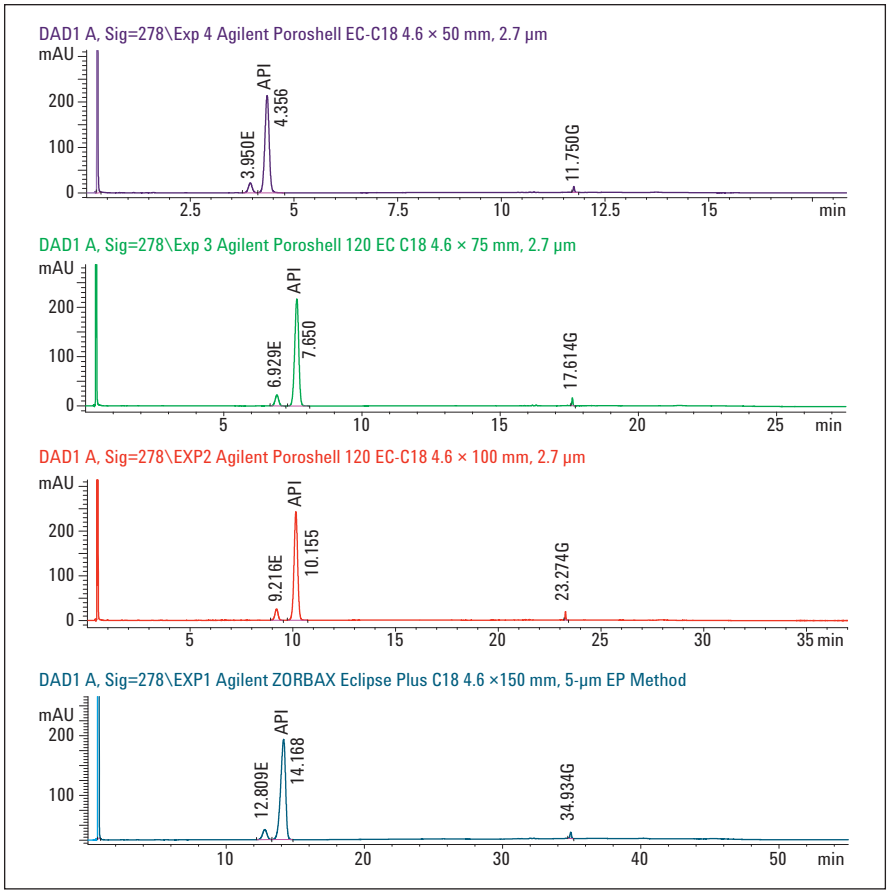

Figure 1
Separation of salmeterol xinafoate system suitability mix EP method and new cost saving methods.

SI no.	Test	Limit	Results Exp 1	Exp 2	Exp 3	Exp 4
1	Peak valley ratio, between impurity E and salmeterol	10	23	70	80	58
2	RRT of impurity E	~0.9	0.9	0.9	0.9	0.9
3	RRT of impurity G	~2.7	2.5	2.3	2.3	2.6
4	RSD RT (salmeterol peak) (%)		0.03	0.00	0.03	0.00
5	RSD Area (salmeterol peak) (%)		0.28	0.23	0.13	0.10
6	Resolution between impurity E and salmeterol		2.18	3.11	2.83	2.24

Table 5
System suitability results obtained from EP method and new cost saving methods.

A 50% reduction in particle size is allowed as a deviation of column dimension. Agilent Poroshell columns with a 2.7-µm particle size were used to develop the new methods (46% deviation). This reduction in particle size helped to better resolve the peaks compared to the original method (Experiment 1). As a proof, increased resolution value between impurity E and salmeterol peaks are tabulated in Table 5. Superior separation achieved for several trace impurities in the system suitability sample mix using Experiment 2 conditions compared to the EP method EP method (Experiment 1) is shown in the Figure 2 insert.

A reduction in column length contributed to saving time and solvent by 33.3%, 50.0% and 66.6% for Experiments 2, 3, and 4, respectively. Adopting Experiment 2 conditions resulted in US $ 26 saving per injection. Total cost saving calculations for Experiments 3 and 4 per injection were found to be US $ 39 and US $ 52 respectively. Figure 3 summarizes the savings in time, solvent and cost per injection.

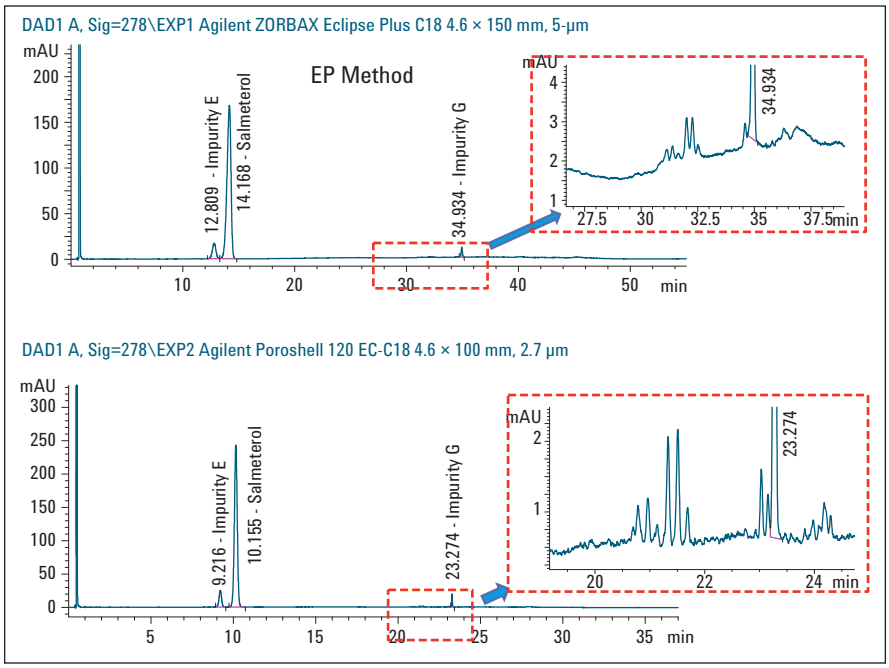

Figure 2
Improved separation of trace impurities in salmeterol xinafoate system suitability mix under Experiment 2 conditions using an Agilent Poroshell column compared to the pharmacopeia method.

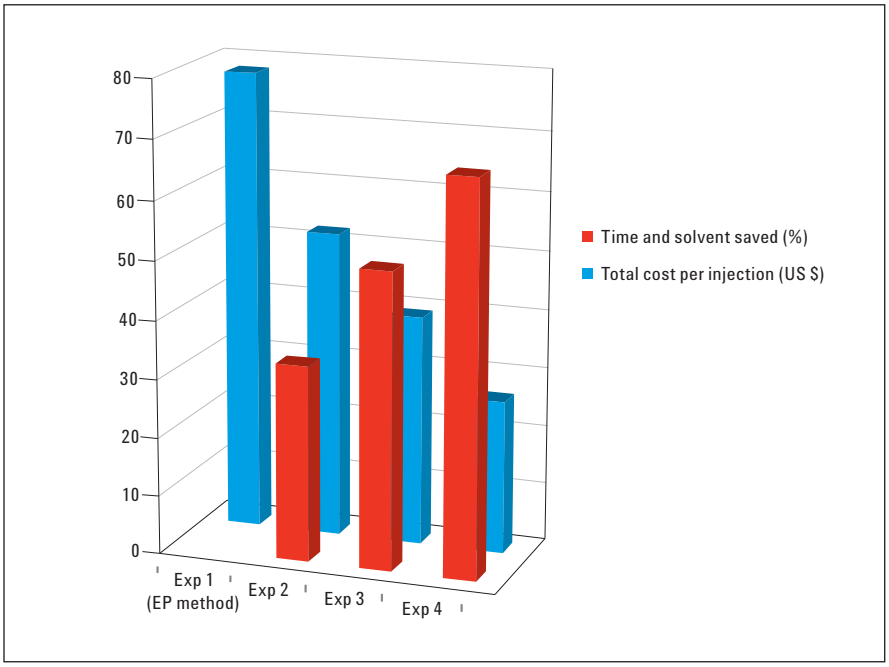

Figure 3
Time and solvent savings and total cost calculation for all the experiments.

Conclusions

- Significant cost reduction for salmeterol xinafoate chromatographic related substance analysis was achieved by modifying column dimensions within the guidelines of EP allowed column deviations.
- Since the deviations incorporated are within the pharmacopeia deviation limit, method revalidation is not needed.
- The method parameters for each modified column dimensions were automatically generated using the Agilent Method Translator. The 1290 Infinity LC System with Intelligent System Emulation Technology (ISET) was used and emulated other instrument configurations for the experiments.
- The system suitability results using the improved methods were within EP's acceptance criteria.
- Poroshell 120 EC C18 columns enhanced the separation of peaks in the system suitability mix.
- By reducing the length and particle size within the allowed deviations, the total cost of analysis per injection can be brought down to US $ 26 from US $ 78 with a 66.6% time saving.

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Published in the USA, May 1, 2012
5991-0394EN

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