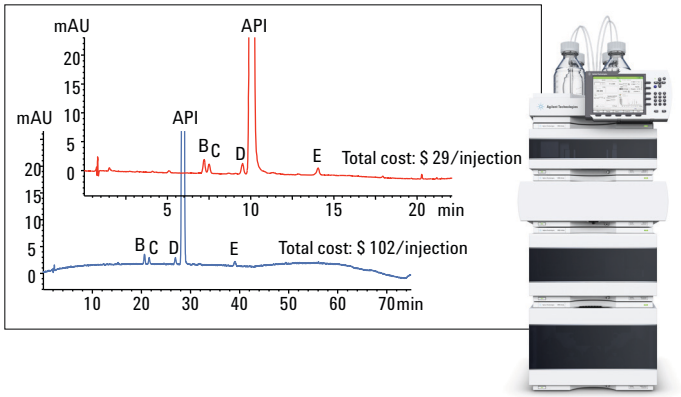
Effective use of Pharmacopeia guidelines to reduce cost of chromatographic analysis for Fluticasone propionate

Application Note

Pharmaceutical QA/QC



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Abstract

The Agilent 1290 Infinity LC System features a quaternary pump with an extended pressure range up to 1200 bars. This pump allows easy, fast and secure transfer of ternary chromatographic methods from conventional columns to smaller column dimensions resulting in higher resolution and or faster separations. This Application Note shows that, by using the example of Fluticasone propionate United States Pharmacopeia (USP) organic impurity method, a significant amount of time, solvent, and costs can be saved by varying column dimensions. The column dimension modifications were within the allowed deviations of USP guidelines, thus eliminated the need for method revalidation. Agilent's Method Translator and Cost Saving Calculator was used to derive new gradient parameters. A 93.8% reduction in solvent consumption and 71.3% reduction in cost of analysis per injection were achieved solely varying column dimensions within USP guidelines.

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Introduction

Fluticasone propionate is a block buster corticosteroid drug used to treat asthma and allergic rhinitis[1]. The USP method for organic impurities of Fluticasone propionate is approximately 75 minutes long and uses a 4.6 × 250 mm column with a 5-µm L1 packing[2]. The mobile phase composition is a ternary solvent mixture. A quaternary pump is the best fit for ternary analysis. Considering a cost of US $ 60/L for acetonitrile, US $ 25/L for methanol, and labor cost of US $ 80/hour for running an instrument, the total cost of analysis for fluticasone propionate amounts to US $ 102.6/injection. This includes the US $ 1.5/L expense for solvent waste disposal. By reducing the column length, diameter, and particle size within the allowed USP deviation limit, a significant reduction in analysis time and solvent consumption can be achieved, resulting in a reduction of total cost of analysis per injection. USP <621> guidelines on permitted column dimension deviations for LC methods are provided in Table 1[3].

In this Application Note, we performed the standard USP organic impurity analysis for fluticasone propionate (Experiment 1) using an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm column. To demonstrate the potential reduction in cost of analysis, three additional methods (Experiments 2, 3, and 4) were performed with modified column dimensions. Since the incorporated column dimension modifications are within the USP allowed deviation limit, a revalidation of the method is not required. However, for the adoption of any column dimension modification, a system suitability test as per pharmacopeia method should be performed. To meet this pharmacopeia requirement, system suitability tests were performed on new column dimensions. An Agilent 1290 Infinity Quaternary LC system was used for the experiments which ensured the ultimate performance of a binary high-pressure mixing UHPLC pump with the flexibility of a low-pressure mixing quaternary pump.

Experimental

Instruments, columns and Software

The individual modules for Agilent 1290 Infinity Quaternary LC System used for the experiment are:

- Agilent 1290 Infinity Quaternary Pump (G4204 A)
- 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)
- The systems was controlled using the Agilent ChemStation Open lab CDS ChemStation Edition C.01.03

Table 2 shows the column parameter details of columns used in all the four experiments.

Column parameter	USP limit for deviation
Length	+ 70%
Internal diameter	No limit, but keep constant linear velocity
Particle size	-50%

Table 1
Allowed column deviations as per USP <621> recommendation

Column parameter	Experiment 1 (USP recommended column dimension)	Experiment 2		Experiment 3		Experiment 4	
		Actual	% Deviation	Actual	% Deviation	Actual	% Deviation
length	250 mm	100 mm	-60	100 mm	-60	75 mm	-70
Diameter	4.6 mm	4.6 mm	0	2.1 mm	-54	2.1 mm	-54
Particle size	5 µm	2.7 µm	-46	2.7 µm	-46	2.7 µm	-46

Table 2
Column dimensions used for various experimental conditions and their deviations from the original USP method. Column packing material used for all experiments was L1.

Reagents and materials

The USP reference standards for Fluticasone propionate system suitability mix was purchased from USP-India Private Ltd, Hyderabad, India. Acetonitrile and methanol were of super gradient grade and were 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. Phosphoric acid was purchased from Aldrich (India).

Chromatographic parameters

The mobile phases were prepared as per the USP method. The mobile phase A was prepared by mixing 0.5 mL of phosphoric acid in 1 L of acetonitrile. Mobile phases B and C were prepared by mixing 0.5 mL each of phosphoric acid in 1 L of methanol and water respectively. The column temperature was maintained at 40 °C and the detection was set at 239 nm. The method parameters for the modified column dimensions were calculated using an Agilent method translator and cost saving calculator. Table 3 shows the detailed chromatographic method parameters used for each experiment.

	Agilent 1290 Infinity Quaternary LC System															
Parameter	**Experiment 1 (USP method)**				**Experiment 2**				**Experiment 3**				**Experiment 4**			
Injection volume:	20 µL*				20 µL				4.2 µL				3.1 µL			
Column:	Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm (p/n 959990-902)				Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-902)				Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)				Agilent Poroshell 120 EC-C18, 2.1 × 75 mm, 2.7 µm (p/n 697775-902)			
Flow rate:	1.0 mL/min				1.0 mL/min				0.21 mL/min				0.21 mL/min			
Gradient:	Time (min)	% A	% B	% C	Time (min)	% A	% B	% C	Time (min)	% A	% B	% C	Time (min)	% A	% B	% C
	0	42	3	55	0	42	3	55	0	42	3	55	0	42	3	55
	40	53	3	44	16	53	3	44	16	53	3	44	12	53	3	44
	60	87	3	10	24	87	3	10	24	87	3	10	18	87	3	10
	70	87	3	10	28	87	3	10	28	87	3	10	21	87	3	10
	75	42	3	55	30	42	3	55	30	42	3	55	22	42	3	55
Acquisition rate:	10 Hz				10 Hz				20 Hz				20 Hz			

Table 3
Chromatographic parameters used for all the four experiments. (*As per USP, injection volume is 50 µL).

Procedure

The fluticasone propionate system suitability mix was prepared as per USP assay method described in USP 35–NF 30[2]. The USP system suitability mixture contains about 99% of fluticasone propionate active pharmaceutical ingredient (API) and about 0.25% each of fluticasone propionate related compound (RC) B, C, D, and E.

The system suitability test for the Fluticasone propionate organic impurities method includes:

a) Measurement of the resolution between fluticasone propionate related Compound B and fluticasone propionate related Compound C using system suitability mix (limit: not less than 0.6)

b) Measurement of the resolution between fluticasone propionate related Compound D and fluticasone propionate using system suitability mix (limit: not less than 1.5)

The system suitability testing for organic impurity method was performed on all four experimental conditions.

Results and Discussion

Separation and detection

Figure 1 shows the separation of Fluticasone propionate and impurities using a standard USP method (Experiment 1). All peaks are well separated and the observed resolution between related Compound B and related Compound C are approximately 2.1. Resolution between fluticasone propionate related Compound D and fluticasone propionate was found to be 2.83.

A similar chromatographic elution profile was achieved using newly developed cost effective methods (Experiments 2, 3, and 4). Figure 2 shows the chromatogram of system suitability test results using the new experimental conditions and Table 4 tabulates the summary of results. The system suitability test results for organic impurities using the new experimental conditions were within the acceptance criteria.

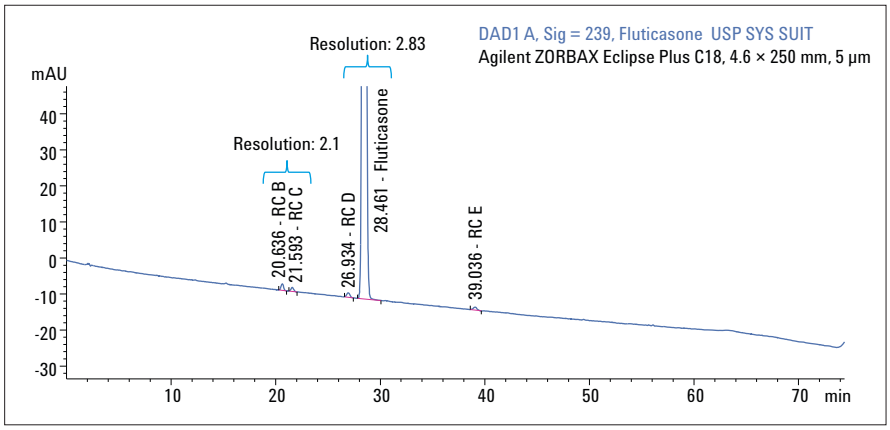

Figure 1
Separation of fluticasone propionate and impurities as per standard USP method.

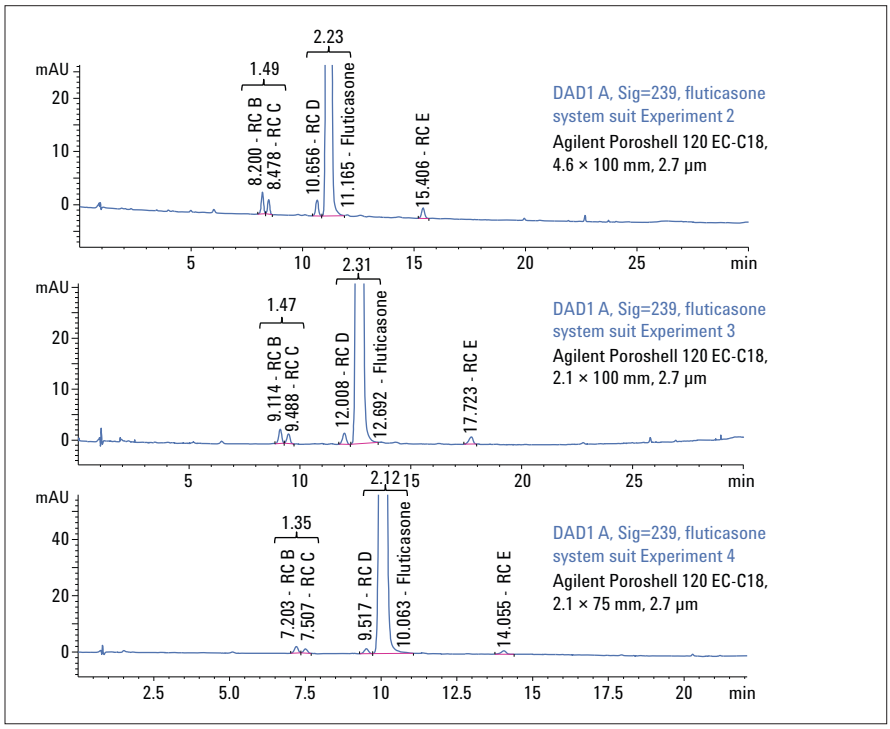

Figure 2

Separation of fluticasone propionate system suitability mix using newly developed cost effective experimental conditions. The resolution values for system suitability testing are marked.

			Result			
Sl no.	**USP System suitability test**	**Limit**	**Exp I (USP)**	**Exp 2**	**Exp 3**	**Exp 4**
1	Resolution between fluticasone propionate related Compound B and fluticasone propionate related Compound C	NLT# 0.6	2.1	1.49	1.47	1.35
2	Resolution between fluticasone propionate related Compound D and fluticasone propionate	NLT#1.5	2.83	2.23	2.31	2.12
3	Relative retention time (RRT) of RC B	0.75	0.73	0.73	0.72	0.72
4	RRT of RC C	0.8	0.76	0.76	0.75	0.75
5	RRT of RC D	0.95	0.95	0.95	0.95	0.95
6	RRT of RC E	1.3	1.37	1.38	1.40	1.40
7	Relative standard deviation (RSD) RT for fluticasone peak (%), n = 5)	NA	0.20	0.06	0.05	0.04
8	RSD area for fluticasone peak (%) (n = 5)	NA	0.31	0.34	0.34	0.17

Table 4

Results summary for all four experiments (NLT = not less than).

Cost of analysis

The cost of analysis was calculated for all the four experimental conditions. The approximate time required to complete one injection for fluticasone propionate USP organic impurity method is approximately 75 minutes. The total analysis cost was calculated to be approximately US $ 102.6/injection. In Experiment Condition 2, the column length was reduced to 100 mm resulting in a 60% time and solvent saving. The estimated analysis cost of Experiment 2 is approximately US $ 41 per injection. In Experiment 3, the column diameter was reduced to 2.1 mm while maintaining the column length as 100 mm and a total solvent saving of 91.6% was achieved. In Experiment 4, further cost reduction was achieved by reducing the gradient time with a shorter 75-mm column while maintaining id as 2.1 mm. The total analysis expense using Experiment 3 and 4 conditions was calculated as US $ 40.1/injection and US $ 29.4/injection respectively. Figure 3 shows the summary of total cost, solvent saving, and time saving calculations for USP and newly developed cost effective experimental conditions.

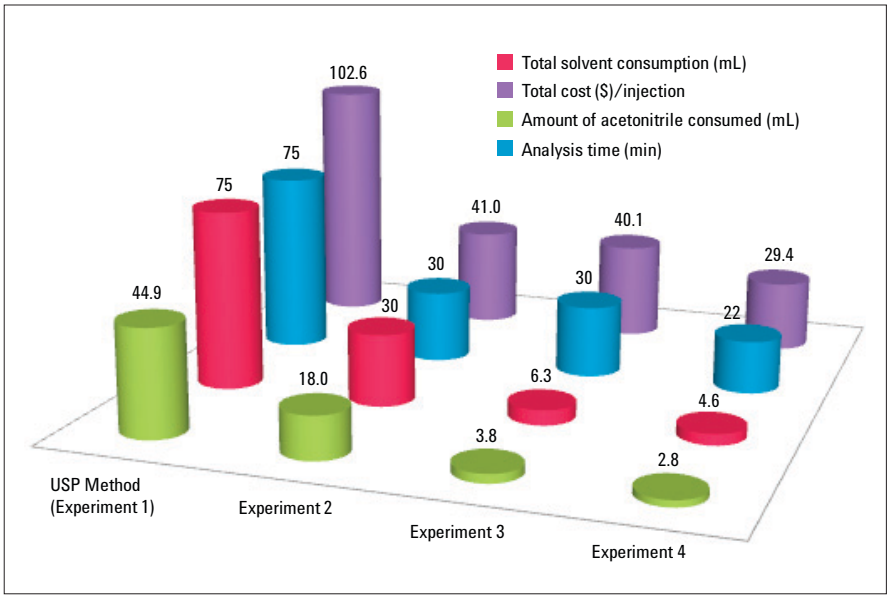

Figure 3
Total cost, solvent saving, and time saving calculations for USP and newly developed cost effective experimental conditions.

Conclusion

This Application Note demonstrates how the effective use of the Agilent 1290 Infinity Quaternary LC System can reduce the total cost of chromatographic separation for a generic drug like fluticasone propionate. The column dimensions recommended by USP were varied within the allowed pharmacopeia deviation to achieve cost reduction without the need for method revalidation. The cost of analysis can be significantly reduced by reducing column dimensions within the pharmacopeia deviation limits.

References

1.
Wikipedia search result for fluticasone propionate

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Published in the USA, January 1, 2013
5991-1602EN

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